VOLUME XXII APRIL, 1939 NUMBER 4

JOURNAL OF DAIRY SCIENCE

Contents

The physiological types of bacteria in milk in relation to reduction of methylene blue. R. N. DAVIS AND K. W. LEWIS 209

Determining the efficiency of ice cream mix pasteurization through the use of the phosphatase test. A. J. HAHN AND P. H. TRACY 219

A study of factors concerning herd management and herd production. LYNN COPELAND 229

The chemical composition of the blood of the dairy goat. O. B. HOUCHIN, W. R. GRAHAM, JR., V. E. PETERSON AND C. W. TURNER 241

Studies on the composition of bovine blood as influenced by gestation, lactation and age. WARARAN L. KENNEDY, ARTHUR K. ANDERSON, S. I. BECHDEL AND J. R. SHIGLEY 251

Certain factors affecting the phosphatase test when applied to ice cream. W. J. CAULFIELD AND W. H. MARTIN 261

Ascorbic acid and oxidized flavor in milk. I. Distribution of ascorbic acid and occurrence of oxidized flavor in commercial grade A raw, in pasteurized irradiated, and in pasteurized milk throughout the year. G. M. TROUT AND ERLAND C. GJESSING 271

Magnesium and vitamin D relationships in calves fed mineralized milk. C. E. KNOOP, W. E. KRAUSS AND C. C. HAYDEN 283

American Dairy Science Association Announcements 291

New Members for 1939 by States 293

Abstracts of Literature A57

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION

Made in United States of America

JOURNAL OF DAIRY SCIENCE

VOLUME XXII APRIL, 1939 NUMBER 4

THE PHYSIOLOGICAL TYPES OF BACTERIA IN MILK IN RELATION TO REDUCTION OF METHYLENE BLUE

R. N. DAVIS AND K. W. LINES
University of Arizona

Since very early in the 20th century various dye reduction reactions have been used as indications of the bacterial count of milk and of its keeping quality. The reductase test has served the dairy industry admirably in providing a cheap, quick, and rather simple and accurate test, for the quality of milk. The results of the methylene blue test, however, cannot be fairly and accurately correlated with the plate count as to numbers of bacteria in the milk. This fact should not limit its usefulness.

HISTORICAL

The chemical basis of the methylene blue oxidation-reduction reaction has been the subject of a good deal of controversy. Likewise the factors involved in reaction have been disputed.

Barthel (1) believed that the disappearance of methylene blue in milk takes place in two stages, *viz.*:

1. The removal of the dissolved oxygen by the growing bacteria.
2. The reduction of the dyes by constituents of the milk. There are considerable data in support of this theory. Thornton and Hastings (7 and 8) reported it is probable that minute but inconsequential amounts of oxygen are fixed by constituents of the milk during the operation of the test. Their work confirms Barthel's theory. Therefore, the reduction time of methylene blue in raw or pasteurized milk may be looked upon as a criterion of the oxygen-consuming power of the bacteria in the milk. Thornton and Hastings (7 and 8) conclude that the reduction times of methylene blue in milk, as observed in the standard operation of their test are influenced almost entirely by the rate of oxygen uptake by the bacteria in the milk.

The rate of oxygen uptake by different species of bacteria are not well known (3, 4 and 6). Hegarty (5) reports that oxygen utilization varies greatly. Experiments reported by Bendixen and Ellington (2) showed that proteolytic, alkali-forming, inert and high lactic-acid-producing organisms reduce milk and cream as effectively as ordinary lactic acid bacteria. The

Received for publication July 28, 1938.

reducing power of different organisms and of the same organisms under different conditions also appeared variable, they found.

It is probable that the rates vary sufficiently with the different species, and with different conditions, to introduce an important factor of inaccuracy into the test. This, Thornton and Hastings believe, is one of two important sources of inaccuracy, the second being the removal of bacteria from the milk by the rising butterfat.

In this paper an attempt is made to show the influence of different physiological types of bacteria on the rate of oxygen absorption and hence on the rate of reduction, and the relative number of the different varieties present at the time of reduction.

EXPERIMENTAL

Samples of milk, drawn by machine, were taken from individual cows at the University of Arizona farm. Samples were also taken of herd milk from other dairy farms. The samples were then subjected to the methylene blue test as prescribed by "Standard Methods of Milk Analysis." At the time of reduction triplicate plates were poured with standard agar in three dilutions. These plates were then incubated at 37° C. for approximately 48 hours. At this time a colony count was made using a hand lens. All plates were counted when possible and an attempt was made to correlate the counts of one plate with another. After counting, the plates were marked off in four different equal portions. The quarter portion having the most evenly distributed colonies was then selected and 25 consecutive colonies from this portion were inoculated into litmus milk, being careful that the colonies were representative of the entire plate. These inoculated litmus milk tubes were then held at laboratory temperature for periods running from 4 to 7 or 8 days, according to the temperature. The temperature of the laboratory seldom goes below 60° F. in the winter or above 90° in the summer. As soon as bacterial action made it possible to detect the physiological types of bacteria in the tubes, the tubes were examined and classified. The tubes were classified as containing acid-coagulating, acid-not-coagulating, acid-coagulating-gas-proteolytic, acid-proteolytic, alkaline, and inert bacteria and a record was also made of the blank tubes in which no action occurred. Blank tubes and tubes containing inert bacteria were determined by making agar slants of the medium.

Accurate records were kept of the date when the samples were taken, the cow or herd from which they were taken, the time required for reduction of methylene blue, the plate count after reduction, and the physiological types of bacteria present. Over 1,000 different samples in all were run, making more than 25,000 tubes that were inoculated.

On about ½ of these samples plate counts were made before subjecting the samples to the methylene blue test and the physiological types of bac-

teria were determined as above to determine which organisms were most active in growth activities in the methylene blue milk.

In all the experimental work, a concerted effort was made to keep all instruments used in a sterile condition and to prevent outside contamination of samples. Sterile 1 cc. and 10 cc. pipettes were used for drawing methylene blue and milk into the tubes. Sterile 30 cc. test tubes were used for making the test and for holding inoculations.

RESULTS

Normal Proportion of Different Types of Bacteria in Milk

In summing up the results of the 25,000 inoculations the results show an average of 6.27 per cent acid-coagulating, 55.82 per cent acid-not-coagulating, 11.59 per cent acid-coagulating-gas-proteolytic, 16.56 per cent acid-proteolytic, 2.68 per cent alkaline, 2.83 per cent inert, and 4.25 per cent blank tubes. This milk was produced under sanitary conditions but came from different farms and was produced under different conditions. The plate count of the milk before reduction varied from about 2,000 bacteria per cc. up to as high as 30,000 per cc. with an average count of about 8,000 per cc. This is thought to be fairly typical of most grade A raw milk produced under the same conditions and the bacterial flora can for all practical purposes be considered to be about the same in all such grade A milk under normal conditions. It was found that the approximate average reduction time for this milk was, in round numbers, about 6 hours, but varied from

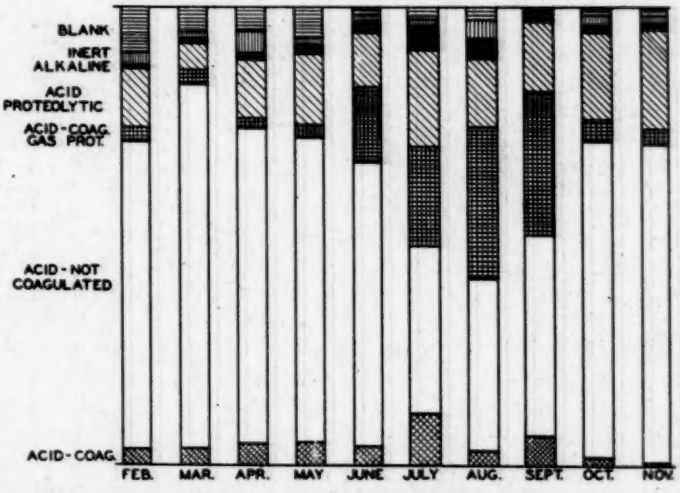

FIG. 1. This shows the wide seasonal variation in types of bacteria. Note the acid-coagulating-gas-proteolytic and acid-not-coagulating types particularly.

about 45 minutes up to as high as 14 and 15 hours in rare instances. The blank tubes are considered as having been inoculated with bacteria which find milk an unsuitable medium for growth, or it may be that the colony may have been missed by the inoculating needle in making the inoculation. Considerable difficulty was encountered with pin-point colonies, especially during the summer months, which made it hard to make certain of inoculations.

Seasonal Variations in Type

It was found (see Figure 1) that during the spring and summer months the number of acid-not-coagulating bacteria constantly decreased until fall.

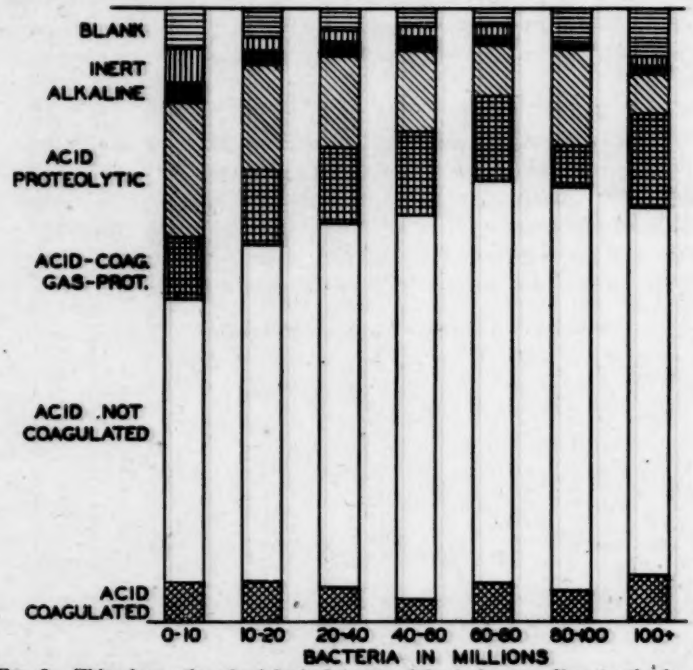

FIG. 2. This shows the physiological types of bacteria according to the bacterial count after reduction.

During this time the number of acid-coagulating bacteria, the alkaline and particularly the acid-coagulating-gas-proteolytic bacteria increased. The blank, inert, and acid-proteolytic types varied considerably from month to month but the variation was not uniform. From September until March the variation in type was exactly opposite. This is thought to be of significance in view of the fact that the average time for reduction decreased during the spring and summer and increased during the winter months, show-

ing approximately the same trend as the acid-not-coagulating types and a trend exactly opposite to that shown by the acid-coagulating, alkaline, and acid-gas-coagulating-proteolytic types.

Relationship between Plate Count after Reduction and Type

The data showed that as the plate count after reduction increased (see Figure 2) the number of acid-not-coagulating bacteria increased, the number of acid-coagulating-gas-proteolytic bacteria increased as a rule and the number of alkaline, inert and acid-proteolytic bacteria decreased. The other types varied but not uniformly. These observations were to quite an extent in agreement with what we had expected in accordance with other observations. No conclusions can be drawn, however, from this particular phase of the study without first taking some other comparisons into consideration. It was noted that 16.87 per cent, 28.44 per cent, 35.05 per cent, 14.34 per cent, 3.23 per cent, 1.22 per cent, and .88 per cent of all the samples observed had from 0–10, 10–20, 20–40, 40–60, 60–80, 80–100 and above 100 million bacteria per cc. respectively, after reduction. The average samples had from 20 to 40 million bacteria per cc. at the time of reduction.

Relationship between Time of Reduction and Type

Bendixen and Ellington (2) observed that the reducing power of different organisms and of the same organism under different conditions appeared variable. This observation was strikingly substantiated when an attempt was made to show any relationship between time of reduction and type. The percentages of the different organisms varied greatly as the time but the variation in no instance showed any particular trend. This was contrary to expectation. This leads us to believe there is an association action between the different types of bacteria, and that the rate of oxygen absorption by the different types of bacteria is very materially influenced by their co-habitation with other types of bacteria. This belief has been substantiated by other workers.

Time-Count Relationships

A complete biometrical study of the data was made in an attempt to find a relationship between reduction time and type, and between count and type. In most instances there was no general trend for any of the types of bacteria, as the bacterial count increased. It was noted, however, that in most instances the acid-not-coagulating type of bacteria showed a tendency to increase and that the acid-proteolytic type tended to decrease as the count increased. This tendency was not constant. For other types, no such tendencies could be found. In a further study it was noted that in the shorter reduction times the acid-not-coagulating types were not prevalent and that the acid-proteolytic types and acid-coagulating types predominated. Just the opposite of this was true in longer reduction times.

On the basis of these time-count type studies of both the count in thousands before reduction and the count in millions after reduction and the physiological types both before and after reduction it was concluded that there is no very close relationship between physiological type and reduction time.

Growth Rates During Reduction Test

In order to see if any specific types were more active in their growth activities than other types, charts[1] were prepared using count classifications in millions after reduction of from 0–5 million, 5–10, 10–20, 20–30, 30–40, 40–50, 50–100, and 100 million and above to show the relationships of percentages of the various physiological types both before and after reduction. A study of this chart showed that in general the percentage of acid-coagulating, acid-proteolytic, alkaline, inert, and blank tubes decreased during the reduction process. The number of acid-not-coagulating and acid-coagulating-gas-proteolytic types increased. The tendency of the acid-coagulating-gas-proteolytic types to increase became less and less as the bacterial count increased, while the tendency on the part of the other types to either increase or decrease remained nearly constant for all bacterial count classifications.

A similar study was made classifying the bacteria both before and after reduction according to time of reduction. Reduction times of from 3 to 4 hours, 4–5, 6–7, 7–8, 8–9 hours and above were used. Increases and decreases of the various types similar to those found in the previous study were observed.

It was noted, however, that as the count went up the percentage of acid-coagulating-gas-proteolytic types increased while the percentage of acid-not-coagulating types decreased. As the time for reduction increased there was an exactly opposite trend, the acid-coagulating-gas-proteolytic types decreased while the number of acid-not-coagulating types increased. This is thought to be significant.

Table 1 shows the proportion of the various types before reduction to the

TABLE 1

Changes of percentages of types during the reduction process

Types of bacteria	Acid-coagulating	Acid-not-coagulating	Acid-coagulating-gas-proteolytic	Acid-proteolytic	Alkaline	Inert	Blank
Before reduction	7.3%	22.9%	31.3%	23.3%	5.8%	5.1%	4.2%
After reduction	1.7	41.5	37.9	12.9	1.6	2.1	2.2

[1] A copy of these charts can be had by writing the authors.

same type after reduction of those samples in which both studies were made. Count and reduction time were not considered in making these comparisons. This table shows increases and decreases in numbers similar to those found in the other studies.

A study of the relationship between the original plate count before reduction and the count after reduction as well as the reduction time was made. It was found that there is no direct relationship between the count before and the count after reduction. There is a relationship, however, between the original count and the time of reduction. As has always been found by other workers, as a general rule the time for reduction varies inversely with the original count. The authors found numerous exceptions to this rule, however.

Table 2 shows the relationship between the time of reduction and the count after reduction. It will be noticed that, as the time for reduction increased the count also increased. This was in accordance with what we had expected on the basis of other studies. It shows clearly that one or more types present must have an inhibitory effect on one or more of the other types. So far we have been unable to show any close relationship between type and reduction time.

TABLE 2

Bacterial count after reduction according to reduction time

Reduction time	Average count after reduction
0– 2 hours	10,000,000 per cc.
2– 3 "	17,000,000 " "
3– 4 "	19,000,000 " "
4– 5 "	21,000,000 " "
5– 6 "	21,000,000 " "
6– 7 "	23,000,000 " "
7– 8 "	22,000,000 " "
8– 9 "	33,000,000 " "
9–10 "	37,000,000 " "
10–11 "	36,000,000 " "
Above 11	61,000,000 " "

Type Variations in Individual Samples

The variation in type found in individual samples was considerable. No close relationship between time of reduction and type was found. It may be concluded that differences in reduction time for different samples bear no direct or great relationship to the types of bacteria present.

DISCUSSION

It seems that there is a very marked seasonal variation in the physiological types of bacteria in milk. Part of this variation may have been due, however, to seasonal variations in the laboratory incubation temperature. The decline in reduction time during the spring and summer months would

indicate that the change is one of type rather than of growth rates due to temperature changes. The temperature change in the laboratory in which the litmus milk tubes were incubated may have played a small part in the physiological type change. Nevertheless, the decrease in the number of acid-not-coagulating type of bacteria which followed the decrease in time of reduction would indicate that acid-not-coagulating types are poor reducers. On the other hand, during the same period in which the acid-not-coagulating types decreased there was an increase in the number of alkaline and acid-coagulating-gas-proteolytic types of bacteria which would indicate that they are primarily important in reducing.

Barthel (1) points out that reduction does not occur until oxygen has been partly absorbed. This would indicate that acid-not-coagulating types of bacteria are poor utilizers of oxygen and that alkaline and acid-coagulating-gas-proteolytic types are rather important in oxygen utilization.

The study shows, however, that acid-not-coagulating types of bacteria predominate in good quality raw milk and so must play a large part in reducing the dye. The acid-coagulating-gas-proteolytic types make up only 11.59 per cent of all bacteria in the samples of this study but as shown above must play a rather important rôle in reduction of methylene blue and oxygen absorption. The small percentage of alkaline bacteria (2.83 per cent) precludes their being of much importance as regards reduction time.

The study shows that as reduction time goes up, the plate count of bacteria after reduction also increases. (See Table 2.) It was also shown that as the count goes up, the acid-not-coagulating types of bacteria increase and proteolytic types of bacteria decrease. This tends to verify the above assumptions that acid-not-coagulating types are slow reducers and oxygen absorbers while proteolytic types are rather hasty oxygen absorbers. (See Figure 2.)

A study of the growth rate variations during reduction, according to bacterial count after reduction again verifies the above assumptions. The acid-not-coagulating types were seen to increase in number as reduction time increased. It is significant, however, that there was an increase in percentage of bacteria of this type after reduction over the percentage before reduction, but that as reduction times increased this tendency decreased. This indicates that if this type of bacteria was present in small amounts before reduction in relation to other types reduction time was short in proportion to the number present. As the number increased before reduction, the time for reduction was increased until at from 9–10 hours for reduction, the number before reduction and after reduction was nearly the same. The important thing, however, is that the acid-coagulating-gas-proteolytic types showed a tendency exactly opposite to that shown by the acid-not-coagulating types. This we think is very substantial evidence as to the absorption and reduction powers of the two types of bacteria.

Since the plate count of the bacteria increases after reduction as reduction time increases (see Table 2) it can be assumed that the bacteria in those samples having a high reduction time are rather inefficient in reducing methylene blue. Acid-not-coagulating types increase as the count after reduction increases.

The time-count comparisons, however, fail to show any tendency for any particular type to be especially important in reducing powers. This, we feel, shows that there is definitely an associative action between bacteria, some types having an inhibitory or possibly an accelerating effect on other types. There was a general tendency for acid-not-coagulating types to increase as time and count increased. The tendency for proteolytic types was exactly opposite. This accelerating and inhibitory effect that co-habitating bacteria have on one another is a common bacteriological phenomenon.

If various types of bacteria do have varying oxygen absorption powers as various workers have found they do (7 and 8), then variations in bacterial flora from individual cows or in herd milks could be assumed to be a source of inaccuracy, when estimating counts on the basis of this test. This study failed to show that such variations are the source of inaccuracy. The associative action among bacteria seems to overshadow any special reducing or oxygen absorptive powers of various physiological types of bacteria.

Since proteolytic types of bacteria have been shown to have some special reducing power, it seems that the ability of the methylene blue test to indicate quality of milk is strengthened. Proteolytic types of bacteria in milk are particularly objectionable especially from the standpoint of cheesemakers and producers of other by-products which must be stored for long periods of time.

SUMMARY

1. Over 1,000 milk samples were studied for physiological types of bacteria present in relation to reduction time of methylene blue.

2. The samples studied were shown to contain on the average of 6.27 per cent acid-coagulating bacteria, 55.82 per cent acid-not-coagulating, 11.59 per cent acid-coagulating-gas-proteolytic, 16.56 per cent acid-proteolytic, 2.28 per cent alkaline, 2.83 per cent inert bacteria and 4.25 per cent of our tubes were blank.

3. There is a wide seasonal variation in physiological types of bacteria in milk.

4. Acid-not-coagulating bacteria are poor reducers of methylene blue but are predominant in most high quality milk.

5. Proteolytic types of bacteria are good oxygen absorbers and play an important part in reduction.

6. There is an associative action between bacteria which usually overshadows individual variations of reduction power.

7. Marked variations in physiological types of bacteria present in individual samples failed to have any material effect on reduction time.

8. Acid-coagulating, alkaline and inert bacteria seem to have no special effect on reduction rates, unless present in unusually large numbers.

REFERENCES

(1) BARTHEL, CHR. Weitere Untersuchungen Uber Die Reduktaseprobe, Sowei Vergleiche Mit einigen Anderen Neveren Milch Hygienischen Untersuchungsmethoden. Zschr. Untersuch. Nahrungsmitt. 24: 137–164. 1917.

(2) BENDIXEN, H. A., AND ELLINGTON, E. V. Grading cream for butter-making. State Coll. Wash. Exp. Sta. Bul. 269. 1932.

(3) BURNETT, F. M. The action of cyanides on bacteria. J. Path. Bact. 30: 21–38. 1927.

(4) COLLUM, A. B. LXIX. The oxygen uptake of bacteria. Biochem. J. 18: 507–518. 1924.

(5) HEGARTY, C. D. Abst. J. Bact. 31: 18. 1936.

(6) SOULE, M. H. The Newer Knowledge of Bacteriology and Immunology. Chap. XVIII. Gas metabolism of bacteria. Univ. of Chicago Press. 1928.

(7) THORNTON, H. R., AND HASTINGS, E. G. Studies on Oxidation-reduction in milk. Oxidation-reduction potentials and the mechanism of reduction. J. Bact. 18: 293–318. 1929.

(8) THORNTON, H. R., AND HASTINGS, E. G. Studies on Oxidation-Reduction in Milk. The methylene blue reduction test. J. DAIRY SC. 13: 221–245. 1930.

DETERMINING THE EFFICIENCY OF ICE CREAM MIX PASTEURIZATION THROUGH THE USE OF THE PHOSPHATASE TEST

A. J. HAHN AND P. H. TRACY
Department of Dairy Husbandry, University of Illinois, Urbana, Illinois

The use of the phosphatase test for determining the efficiency of milk pasteurization has been studied by Kay and Graham (1), Gilcrease and Davis (2), Hahn and Tracy (3) and others. However, the literature contains no reference to the application of this test to ice cream. Since ice cream is a mixture made from cream, milk, condensed milk or milk powder, sugar, gelatin, coloring and flavoring, it does not necessarily follow that the results of the phosphatase test on ice cream would be comparable to those obtained on milk.

The possible combinations of ingredients used in making a mix are unlimited. It was necessary, therefore, for the purposes of this study to select a standard mix from the standpoint of both composition and ingredients used. Unless otherwise specified, the milk products used were raw cream, raw skim milk, and milk powder. All mixes contained 12 per cent fat, 10 per cent serum solids, 15 per cent sucrose and 0.3 per cent gelatin, except in those cases where the nature of the experiment was such that it was necessary to deviate from this standard. In an earlier study by the authors (3), homogenization by pressure was found to have no relation to the phosphatase test, and since it facilitated matters to do so, homogenization was omitted in the preparation of the experimental mixes. All mixes were pasteurized in the laboratory.

The Scharer Test was used to determine the phosphatase content. This test is based on the measurement of the amount of phenol liberated by the action of the phosphatase enzyme on an excess of di-sodium phenyl phosphate during an incubation period of one hour at 37.5° C. The amount of phenol liberated was determined colorimetrically by comparing with known phenol standards. A photo-electric procedure, explained in detail in an earlier publication of the authors (3) was used to measure color intensities of the phenol solutions. For this procedure a series of phenol standards (0.5 to 10.0 p.p.m.) were prepared. These were stored in a dark room at 50° F. for a period of one hour before being used. A glass sample cell was filled with double distilled water and placed in the photoelectric unit and the amount of deflection obtained on a galvanometer. The sample cell was then emptied of the water and filled, in turn, with each of the standard phenol solutions, and the deflection measured. The percentage deflection was calculated according to the following formula:

Received for publication October 6, 1938.

$$\text{Percentage deflection of phenol solution} = \frac{\text{Deflection of water solution} - \text{Deflection of phenol solution}}{\text{Deflection of water solution}}$$

The values obtained were plotted on a graph with their corresponding values in parts per million phenol, the deflection percentage being plotted on the ordinate and the parts per million of phenol on the abscissa. A smooth curve was obtained which represents the calibration curve of the photoelectric unit.

In determining color intensities of unknown solutions, the same procedure was followed, substituting the unknown solutions in place of the phenol standard solutions. The percentage deflection of the unknown solution was determined and the corresponding parts per million phenol interpolated by using the calibration curve.

THE RELATION OF TEMPERATURE OF PASTEURIZATION TO PHENOL VALUE

Six lots of mix were pasteurized at temperatures varying from 143° to 160° F. Samples were taken at the end of 15, 20, 25 and 30 minutes and tested in triplicate. Typical results of a series of tests are given in Table 1.

TABLE 1

Time and temperature of pasteurization as related to the phenol value

Holding period	Temperature of pasteurization					
	143° F.	145° F.	147° F.	149° F.	150° F.	160° F.
	Parts per million phenol					
Raw	3.66	3.66	3.66	3.66	3.66	3.66
15 minutes	3.50	2.65	2.26	1.09	1.10	0.48
20 minutes	3.34	2.60	1.97	0.68	0.61	0.41
25 minutes	3.23	2.47	1.64	0.47	0.40	0.40
30 minutes	3.10	2.38	1.33	0.33	0.32	0.32

These data are of particular interest from the standpoint of the relative effect of the different pasteurizing temperatures at different time intervals. Temperatures of 143° F. and 145° F. inactivated to only a small degree the amount of phosphatase present in the mix. At 147° F. there was a noticeable increase in the inactivation of the enzyme while at 149° to 160° F. the inactivation was what might be considered complete, with no appreciable differences in the value obtained at the end of the 30-minute holding period. However, slight discrepancies in the holding period would likely be more noticeable at 149° and 150° than at 160° F.

EFFECT OF USING PARTIALLY PASTEURIZED INGREDIENTS UPON PHENOL VALUE

Two different types of the standard mix were made. One mix was prepared from milk products that were in a fresh, raw state, and the other mix was made from milk products which had been heated to a temperature of 150° F. for 15 minutes. A set of each of these mixes was heated at 143°,

150° and 160° F. Samples were taken at the end of 15, 20, 25 and 30 minutes and tested in triplicate. Typical results of a series of tests are given in Table 2.

TABLE 2

Effect of using raw or partially pasteurized ingredients in the mix as related to the phenol value

Holding period	Temperature of pasteurization					
	143° F.		150° F.		160° F.	
	Raw	Part past.	Raw	Part past.	Raw	Part past.
Starting mix	3.65	0.91	3.65	0.91	3.65	0.91
15 minutes	3.48	0.90	0.95	0.66	0.51	0.36
20 minutes	3.40	0.75	0.66	0.62	0.40	0.34
25 minutes	3.21	0.65	0.43	0.41	0.32	0.32
30 minutes	3.10	0.61	0.31	0.31	0.31	0.31

It is interesting to note the extreme difference in the final phenol value of the mix made from raw milk products and that mix which contained partially pasteurized milk products, when these mixes were pasteurized at 143° F.

Apparently the presence of protective solids in the ice cream mix has a prohibitive effect on the inactivation of the phosphatase enzyme. The heat treatment to which a mix is subjected when pasteurizing at temperatures of 143° F. is not sufficient to overcome this protective effect, and therefore, incomplete inactivation of the enzyme results. Referring to Table 1, it is evident that the same condition would exist when pasteurizing mix at temperatures as high as 147° F. When pasteurizing at temperatures of 150° F. or above, the heat treatment apparently was sufficient in all cases to overcome the protective effect of the mix solids, since no variations were obtained in the final phenol value of the mix made from all raw milk products and that mix made from partially pasteurized milk products.

COMPARISON OF PHENOL VALUE OBTAINED ON MIX AND MILK SUBJECTED TO THE SAME HEAT TREATMENT

A batch of standard mix made from all raw milk products and the same volume of raw four per cent milk were pasteurized at three different temperatures. Samples were taken after 10, 20 and 30 minutes. Tests were run in triplicate, the phenol values being given in Table 3

Subjecting milk and ice cream mix to the same heat treatment apparently had no varying effect at temperatures above 150° F., as in both cases approximately the same final phenol value was obtained. At a temperature of 143° F., it is evident that the heat treatment was not sufficient to inactivate the enzyme present in the mix to the same extent as that present in the milk due to the protective action of the additional solids present in the mix.

TABLE 3

Comparison of mix and milk as related to the phenol value

Holding period	Temperature of pasteurization					
	143° F.		150° F.		160° F.	
	Mix	Milk	Mix	Milk	Mix	Milk
	Parts per million phenol					
None (control)	3.80	4.50	3.80	4.50	3.80	4.50
10 minutes	2.95	2.11	1.26	0.68	0.46	0.37
20 minutes	2.85	1.10	1.54	0.50	0.40	0.36
30 minutes	2.65	0.73	0.32	0.35	0.32	0.32

EFFECT OF THE CONDENSING OPERATION UPON THE PHOSPHATASE CONTENT OF CONDENSED MILK

Since condensed products are widely used in making ice cream mixes, the effect of the condensing operation on the phosphatase content of milk would be of interest. In order to determine this effect, the phenol value on several batches of condensed milk was determined. These products were pasteurized at temperatures ranging between 143° and 150° F. for 30 minutes before entering the vacuum pan. The condensing process was carried on at temperatures between 140° and 145° F., and the time in the pan was not less than 40 minutes. The lowest value obtained on a finished product was 0.25 parts per million phenol and the highest value, 0.37 parts per million. In Table 4 are given the results of the two high and low batches. Composition of condensed skim milk was 32 per cent total solids and condensed whole milk, 8 per cent fat, 20 per cent milk-solids-not-fat.

TABLE 4

Effect of the condensing process as related to the phenol value

Product	Pasteurizing temp.	State of milk product			
		Raw	After pasteurization	After condensing	Last drawn from pan
		Parts per million phenol			
Skim milk	150° F.	4.00	0.31	0.25	0.25
Whole milk	143° F.	4.22	0.65	0.37	0.37

The results obtained indicate that the condensing process appreciably reduced the phosphatase content of the milk product.

EFFECT OF THE SERUM SOLIDS CONTENT OF THE MIX ON PHENOL VALUE

Three batches of mix were prepared, using as milk products fresh raw cream 30 to 40 per cent fat, fresh raw skim milk, and skim milk powder. The mixes were made so as to have the following composition:

1. 12 per cent fat, 9 per cent serum solids, 15 per cent sugar, 0.30 per cent gelatin.

2. 12 per cent fat, 12 per cent serum solids, 15 per cent sugar, 0.30 per cent gelatin.

3. 12 per cent fat, 15 per cent serum solids, 15 per cent sugar, 0.30 per cent gelatin.

All mixes were heated to 150° F. and samples were taken after 15, 20, 25 and 30 minutes. Samples were tested in triplicate, the results being given in Table 5.

TABLE 5

Effect of the amount of serum solids in the mix as related to the phenol value

Holding period	Per cent serum solids		
	9%	12%	15%
	Parts per million phenol		
15 minutes	0.40	0.43	0.47
20 minutes	0.36	0.39	0.39
25 minutes	0.34	0.35	0.36
30 minutes	0.31	0.31	0.32

When pasteurized at 150° F. for 30 minutes the percentage of serum solids in the ice cream mix had no effect on the final phenol value. It was found advisable, when testing mix of high serum solids content, 12 per cent and above, to use 0.15 cc. instead of the usual 0.10 cc. of lead acetate to completely precipitate the protein.

EFFECT OF STORING MIX ON PHENOL VALUE

Two batches of pasteurized, unflavored and uncolored ice cream mix, one 12 per cent fat, and the other 16 per cent fat, were stored at 40° F. for a period of two weeks, and a phosphatase test made on each batch periodically. All samples were run in triplicate. Phenol values are given in Table 6.

TABLE 6

Effect of storing ice cream mix at 40° F. as related to the phenol value

Mix	Start	Period of time in days								
		1	2	3	4	5	7	11	13	15
		Parts per million phenol								
12% fat	0.38	.38	.37	.37	.37	.36	.34	.34	.35	.35
16% fat	0.34	.34	.34	.34	.35	.33	.31	.31	.32	.32

Storing ice cream mix at 40° F. for a period of two weeks did not appreciably alter the phenol value. In case of the 12 per cent mix a reduction in phenol value of 0.03 parts per million occurred, while the 16 per cent mix reduced 0.02 parts per million. Such small reductions are within range of experimental error and, therefore, are irrelevant in this case.

EFFECT OF FREEZING UPON PHENOL VALUE OF MIX

A batch of mix was pasteurized at 150° F. and sample portions taken at the time the temperature reached 150° F. and again after holding for 15, 20, 25 and 30 minutes. The unflavored and uncolored mixes were aged for two days at 40° F., frozen in a hand freezer and stored at −20° F. The ice cream was sampled after the first and fourth days, and the first, third, eighth and twelfth week of storage. Before testing, the samples of ice cream were heated in a water bath to 100° F. and thoroughly mixed so that as much air as possible could be eliminated. Tests were run in triplicate. The data are given in Table 7.

TABLE 7

Effect of storing mix and ice cream as related to the phenol value

Period of test	Raw	Period of pasteurization at 150° F.				
		0 min.	15 min.	20 min.	25 min.	30 min.
	Parts per million phenol					
Fresh mix	3.66	3.17	0.90	0.62	0.43	0.31
Mix aged 2 days at 40° F.	3.40	2.95	0.88	0.62	0.44	0.35
Ice cream from freezer	3.40	2.95	0.91	0.62	0.43	0.315
At −20° F. for 1 day	3.10	2.65	0.90	0.61	0.43	0.31
" " " 4 days	3.10	2.65	0.88	0.61	0.44	0.32
" " " 1 week	3.10	2.65	0.82	0.62	0.44	0.32
" " " 3 weeks	2.80	2.55	0.88	0.60	0.43	0.34
" " " 8 weeks	2.65	2.41	0.80	0.58	0.41	0.31
" " " 12 weeks	2.63	2.40	0.75	0.56	0.42	0.31

Storing raw mix at 40° F. for two days decreased the phenol value. The same was true for the sample heated to 150° F. but not held. Samples of mix taken at the end of 15 minutes or longer, during the holding period, showed no appreciable change in the phenol value after two days' storage at 40° F.

Storing ice cream at −20° F. over a period of 12 weeks resulted in marked decreases in the phenol values of all the samples except those taken after 20, 25 and 30 minutes in the holding period. The underpasteurized samples after 12 weeks' storage still gave a strong test for phosphatase.

EFFECT OF FLAVORING MATERIALS ON THE ACCURACY OF THE PHOSPHATASE TEST

Five different types of vanilla flavoring materials were added to pasteurized ice cream mix and these samples stored at 40° F. After a period of 1, 3, 7 and 14 days, a phosphatase test was run on the samples. The results of this experiment are shown in Table 8.

Pure vanilla had no effect on the phenol value while the imitation and artificial compounds increased the phenol value to a small degree. Coumarin, however, increased the phenol value approximately 15 times and vanillin showed an increase of double the control sample.

TABLE 8

Effect of vanilla flavoring on the phenol value of ice cream mix

Vanilla	Storage period at 40° F.			
	1 day	3 days	1 week	2 weeks
	Parts per million phenol			
Control	0.31	0.31	0.31	0.31
Pure	0.31	0.30	0.30	0.30
Imitation compound	0.38	0.36	0.36	0.36
Pure and vanillin	0.36	0.35	0.35	0.355
Vanillin	0.60	0.53	0.53	0.52
Coumarin	4.50	4.00	3.60	2.81

In further experiments six different flavoring extracts were added to pasteurized mix and the samples were stored at 40° F. Tests were made on these samples after one, three and seven days in storage. The results are shown in Table 9.

TABLE 9

Effect of the addition of flavor extracts to ice cream mix as related to the phenol value

Extract	Storage period at 40° F.		
	1 day	3 days	1 week
	Parts per million phenol		
Control	0.31	0.31	0.31
Pineapple	0.315	0.315	0.31
Orange	0.325	0.32	0.32
Cherry	0.32	0.32	0.32
Lime	0.32	0.32	0.315
Strawberry	0.34	0.34	0.33
Maple	0.32	0.32	0.35

In five out of six cases the phenol value was increased with the addition of flavor extracts; however, the increase was too small to be considered significant.

EFFECT OF "COLD-PAK" FRUITS UPON PHENOL VALUE

Three different "Cold-Pak" fruits were added to ice cream mix which was then stored at 40° F. Tests were made after one, three and seven days' storage. The flavored samples all soured after one week. Results of this study are shown in Table 10.

TABLE 10

Effect of the addition of "cold-pak" fruits to ice cream mix as related to the phenol value

Fruit	Storage period at 40° F.		
	1 day	3 days	1 week
	Parts per million phenol		
Control	0.30	0.30	0.30
Cherries	0.40	0.42	0.50
Strawberries	0.41	0.41	0.44
Pineapple	0.40	0.41	0.47

It is interesting to note the increase in phenol value in the fruit ice creams during the storage period. This increase may have been caused by bacterial action on the ice cream, resulting in the liberation of phenolic compounds.

EFFECT OF THE ADDITION OF COLOR UPON THE PHENOL VALUE

Numerous experiments were performed to determine the effect of the addition of color to the ice cream mix upon the phenol value. The results obtained varied to such an extent that no definite conclusions could be made.

The coloring material itself had a tendency to be precipitated with the protein on the addition of lead acetate to the substrate-ice cream mixture. If the entire amount of coloring material present was removed in this manner, the phenol value obtained was the same as that of the control sample. However, the precipitation of the color with the protein material by lead acetate cannot be controlled by the operator and in some cases, some of the coloring material passes into the clear filtrate with the resultant increase in the color intensity. Although in several cases the addition of coloring to ice cream mix had no effect on the phenol value, this was not always true. The fact cannot be overlooked that there exists a possibility of incomplete removal of the coloring material with the lead acetate-protein precipitate, so that the use of the phosphatase test to determine pasteurization efficiencies of ice cream to which a coloring material has been added may produce erroneous results.

APPLICATION TO COMMERCIAL ICE CREAM SAMPLES

In order to check the efficiency of pasteurization of ice cream being sold commercially, samples of unflavored ice cream mix were collected from eight different ice cream distributors. A phosphatase test was run on each, the results being given in Table 11.

TABLE 11

Results of phosphatase test on commercial samples

Distributor	Phenol value in parts per million
1	0.32
2	0.31
3	0.32
4	0.32
5	0.28
6	0.35
7	0.31
8	0.29

In all cases but one (Distributor 6) the phenol values obtained were 0.32 parts per million or below, indicating complete pasteurization of the mix had taken place.

SUMMARY

1. Using the phosphatase test, as modified by Scharer, it was found possible to detect variations in the time and temperature of pasteurization of ice cream mixes, provided they had not been colored or flavored.

2. The use of all raw or partially pasteurized milk products in building a mix had no effect on the final phenol value, if the mix was pasteurized at temperatures of 150° F. or above. However, at temperatures of 147° F. or below, the mix made from all raw milk products gave a considerably higher final phenol value than in the case of the mix made from partially pasteurized milk products.

3. Subjecting raw milk and ice cream mix made from all raw milk products to the same heat treatment by heating to temperatures of 150° F. or above and holding for 30 minutes resulted in the same final phenol value for both the milk and mix. Temperatures below 150° F. resulted in a higher final phenol value for the ice cream mix than that of the milk.

4. The condensing process decreased the amount of phosphatase enzyme present.

5. Increasing the serum solids content of the mix had no effect on the final phenol value when the mix was pasteurized at 150° F. for 30 minutes.

6. Storing pasteurized, unflavored and uncolored ice cream mix at 40° F. for a period of two weeks did not appreciably alter the phenol value.

7. Storing unflavored and uncolored ice cream at – 20° F. over a period of twelve weeks resulted in marked decreases in the phenol values of all the samples except those made from mix samples taken 20, 25 and 30 minutes after reaching 150° F. The samples heated for a period of time less than 20 minutes, however, still showed sufficient phenol content for them to be considered underpasteurized.

8. Pure vanilla had no effect on the phenol value while artificial and imitation vanilla compounds caused a slight increase and coumarin and vanillin caused appreciable increases in the phenol value of the mix.

9. Flavoring extracts (other than vanilla) added to the mix did not appreciably alter the phenol value, while the addition of "Cold-Pak" fruits increased the phenol value.

10. The addition of coloring material to ice cream mix in many instances affected the phenol value unless the coloring matter was precipitated with the protein upon the addition of the lead acetate. This, however, was not always possible.

11. Tests run on commercial samples of ice cream mix gave indication of complete pasteurization in all but one case.

CONCLUSIONS

Within certain limits the Scharer Test can be applied as a check on the efficiency of the pasteurization of ice cream mix.

The necessity of establishing standard phenol values for complete pasteurization at various temperatures was definitely shown. Pasteurizing ice cream mix at temperatures of 147° F. or below may result in variable phenol values. This is due to the fact that variation in the heat treatment previously given the dairy products from which the mix is made will cause variation in the amount of phosphatase that survives pasteurization. It is evident that the heat treatment, when pasteurizing mix at temperatures of 147° F. or below, is not sufficient to result in any appreciable inactivation of the enzyme. Apparently the additional solids in the ice cream mix act as a protective agent for the phosphatase enzyme so that more heat is necessary to destroy the enzyme than in the case of milk pasteurization. The possibility that the mix solids act as a protective agent against destruction of bacteria by heat might follow in the same line of reasoning so that the question arises as to whether temperatures of 147° F. or below are sufficient for the proper pasteurization of ice cream mix.

Since coloring and flavoring material affect the accuracy of the phosphatase test, it is evident that some modification will have to be made in the test before it can be used on any unknown sample of ice cream.

REFERENCES

(1) KAY, H. D., AND GRAHAM, W. R. The phosphatase test for pasteurized milk. J. Dairy Res. 6: 191–203. 1935.

(2) GILCREASE, F. W., AND DAVIS, W. S. Investigation of the amylase and phosphatase tests as an indication of pasteurization. Annual Proceedings of the International Association of Milk Sanitarians, 15–32. 1936.

(3) HAHN, A. J., AND TRACY, P. H. Controlling milk pasteurization efficiency through the use of the phosphatase test. J. DAIRY SC. 22: 191–200. 1939.

A STUDY OF FACTORS CONCERNING HERD MANAGEMENT AND HERD PRODUCTION

LYNN COPELAND

American Jersey Cattle Club, New York City

It is becoming a recognized fact that too often in the past breeders have secured official production records on their cows with the primary object of using those records as a means of selling bull calves and surplus breeding stock. We have not studied the individual records on our cows nor have we analyzed them sufficiently with the thought of improving our own herds nor have we as individual breeders concerned ourselves enough with the improving of our respective breeds.

Every official production record on a registered cow of any breed possesses many values. First, it indicates the economic worth of the cow as a producing unit in the herd. Then a production record on an animal furnishes some information as to her transmitting ability to her sons and daughters. Furthermore, the record of the cow assists in proving both her sire and her dam. Likewise the butterfat percentage for the lactation and the persistency of production are valuable for study with the view of maintaining or increasing the average test of the herd and in improving persistency. Also, good production records possess considerable advertising and publicity value and such records add to the sale price of both the cow and her progeny. In addition to the individual records, the information each year on the herd average including the average fat percentage of the herd, breeding efficiency, length of dry periods, services required per cow and the average age of the herd are all of value to the breeder who will study and use such data in trying to do a really constructive work of breeding better dairy cattle.

Herd Improvement Registry records are particularly valuable for herd improvement, due to the fact that every cow in the herd is included and in addition information is available on the herd average each year. To January 1st, 1938, a total of 1046 Jersey herds had completed a year's work of official Herd Improvement Registry testing. These herds included a total of 27,364 cows, of which number 12,274 have finished complete lactation records. The individual lactation records completed by these cows and the information on the herd averages when summarized and tabulated show facts that are of interest and value to every breeder of dairy cattle. It is the object of this paper to present some of the material summarized from these Herd Test records and to attempt to draw such conclusions as seem evident from the data. The paper will be observed to lack continuity in that it does not deal entirely with one subject but instead many different

Received for publication October 24, 1938.

observations that seemed to be of interest and of value have been summarized and are presented.

Quite often the term, breeding efficiency, has been used to denote the number of calves per cow, per year, and formulas have been published illustrating how the breeding efficiency of a herd may be computed on this basis. However, in this paper liberty has been taken to use the term, breeding efficiency, as indicating the fraction of the year which a cow is in milk or inversely the percentage of the herd dry during the year. This of course is definitely related to the number of calves dropped during the year although persistency of production may also influence it.

In observing the data on the herd averages as completed each month, over a period of years, it seemed evident that breeding efficiency and the average production of the herd were directly related. On the certificate which a breeder receives at the end of the Herd Test year and also in the annual record volumes, two figures are given which indicate in a large measure how efficiently the herd has been operating from a breeding and calving standpoint. One figure shows the average number of cows in the herd and the other figure shows the average number of cows in milk throughout the year. The difference between these two figures represents the average number of days that each cow was dry during the year. Table 1 shows the relationship between the breeding efficiency and herd averages of the 1046 herds that had completed a year's work of herd testing prior to January 1st, 1938.

TABLE 1

Effect of breeding efficiency on herd average

Production divisions	Number of herds	Percentage of herd dry	Average number of days each cow was dry
Below 250 lbs. fat	40	27.6	101
250 to 299 lbs.	119	18.8	69
300 to 349 lbs.	248	15.9	58
350 to 399 lbs.	344	13.3	49
400 to 449 lbs.	190	11.5	42
450 to 499 lbs.	60	10.5	38
500 lbs. and over	45	10.4	38
Totals (All herds)	1046	14.5	53

The coefficients of correlation existing between the herd averages of the 1046 herds and the percentage of dry cows in each herd was determined and found to be $-.57 \pm .014$. This correlation is sufficiently high to be significant and together with the data in the table indicates that the percentage of dry cows in the herds on Herd Test is inversely proportional to the herd average. In fact it seems that the low average yield of many herds is not due entirely to low inherited producing ability but that perhaps breeding troubles and management were also largely responsible. The carrying

TABLE 2

Frequency table showing percentage of cows dry in herds of different levels of production

Production divisions	Percentage of herd dry									
	Under 5%	5 to 9%	10 to 14%	15 to 19%	20 to 24%	25 to 29%	30 to 34%	35 to 39%	40% and over	Totals
Under 250 lbs.	0	0	3	3	11	7	9	3	4	40
250 to 299 lbs.	0	8	26	38	30	9	5	2	1	119
300 to 349 lbs.	0	17	106	78	35	9	1	2	0	248
350 to 399 lbs.	9	67	173	77	13	4	1	0	0	344
400 to 449 lbs.	5	58	88	39	0	0	0	0	0	190
450 to 499 lbs.	5	21	28	6	0	0	0	0	0	60
500 lbs. and over	4	17	21	3	0	0	0	0	0	45
All herds	23	188	445	244	89	29	16	7	5	1046

of old cows, long past their prime in production, in the hope of getting another calf or two is customary in some herds. This, however, can hardly be responsible for the high percentage of dry cows in the low producing herds for such brood cows are usually carried only in the better herds.

The average percentage of dry cows in all the herds was found to be 14.48 per cent. It may be that this percentage is somewhat too low for quite often when entering a herd on test for the first time, the owner starts the work when the majority of the cows in the herd have just freshened and in view of this, the percentage of dry cows during the first year's work may be lower than usual. It was found that a total of 368 herds had completed three or more years' work of herd testing and it was observed that the percentage of dry cows in these 368 herds during the third year's work was 15.51 per cent or each cow was dry an average of 57 days or for about eight weeks during the year. This average percentage is a good yardstick by which any breeder may compare the breeding efficiency of his own herd with that of other purebred herds.

Table 2 shows a frequency chart illustrating in a different manner just how important breeding efficiency is to average herd production. In examining this table, it will be observed that not one of the 146 herds with a percentage of dry cows of twenty per cent or more has completed a herd average of above 400 pounds of butterfat and only eighteen of the 146 herds carrying twenty per cent or more dry cows have herd averages of above 350 pounds of butterfat. Furthermore, of the 40 herds averaging under 250 pounds, 16 carried 30 per cent or more of the cows dry during the year.

The size of the herd also affects breeding efficiency and from the data in Table 3, it appears that it is easier to maintain the breeding efficiency of

TABLE 3

Breeding efficiency as affected by size of herd

Size of herd	Number of herds	Percentage of herd dry	Herd averages (lbs. fat)
Less than 10 cows	228	12.7	391.46
10 to 19 cows	472	14.3	364.32
20 to 29 cows	209	15.1	361.47
30 to 39 cows	60	16.0	369.63
40 cows and over	77	17.7	338.77
All herds	1046	14.5	368.03

a small herd than it is in the case of a larger one. This result was not unexpected for it is realized that most of the small herds are owner operated, whereas large herds are usually cared for entirely by hired labor. Furthermore, disease control becomes increasingly difficult as the size of the herd increases.

It is common knowledge that any herd regardless of the herd average

will show a considerable range in the producing ability of the various members of the herd. To determine just what this range was with the Jersey herds doing herd testing, a tabulation was made of these herds. The accompanying graph (Figure 1) shows the range in yield of the cows in these

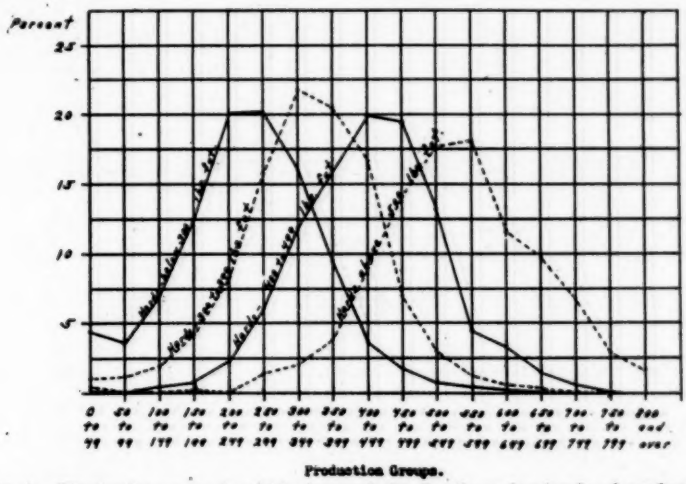

FIG. 1. Illustrating range in yield of cows in herds of varying levels of production.

herds. It will be observed that herds of widely different levels of production show very similar frequency curves in the distribution of the cows on the basis of production. In other words, even those herds averaging over 400 pounds of butterfat usually contain cows with a production very much lower than the average. The chart shows that the amount of variation in production in the high producing herds is as great as in herds of lower levels of production and demonstrates that regardless of the herd average, rigid culling may usually be practiced with advantageous results.

For years all of the Breed Associations have published average production figures of cows on Register of Merit and Advanced Registry test. Such averages however, are based on selected animals and such cows have usually been given better than average attention in regard to feeding and management during the making of the records. Furthermore, the Register of Merit and Advanced Registry requirements have automatically eliminated from such data the production records of low producing animals. In view of this, the information on the averages of the individual Herd Test lactation records is especially pertinent at this time. These records have been summarized depending upon the length of the lactations and the ages of the cows. The summaries are presented in Table 4.

TABLE 4

Herd improvement registry lactation record averages

Length (days)	No. of records	Aver. milk	Aver. fat per cent	Aver. fat	No. of records	Aver. milk	Aver. fat per cent	Aver. fat
	Yearlings				Junior-two-year-olds			
180 or less	17	1608	5.111	82.18	41	1966	5.193	102.10
181 to 210	10	2602	5.108	132.90	18	3148	5.584	175.78
211 to 240	8	2980	5.373	160.13	39	3709	5.173	191.87
241 to 270	59	4220	5.308	223.98	132	4624	5.302	245.16
271 to 305	147	5043	5.347	269.64	420	5511	5.340	294.27
306 to 335	150	5796	5.419	314.07	482	6460	5.299	342.29
336 to 365	261	6479	5.415	350.82	760	7168	5.412	387.95
Totals	652	5564	5.390	299.90	1892	6220	5.359	333.30
	Senior-two-year-olds				Junior-three-year-olds			
180 or less	21	2157	5.170	111.52	24	2285	5.186	118.50
181 to 210	9	3006	5.086	152.89	25	3812	5.538	211.12
211 to 240	35	3899	5.245	204.49	58	4544	5.333	242.34
241 to 270	75	4842	5.262	254.81	189	5432	5.338	289.94
271 to 305	217	5629	5.390	303.38	374	6152	5.335	328.22
306 to 335	203	6688	5.486	366.90	292	7175	5.326	382.15
336 to 365	358	7460	5.446	406.29	436	7815	5.413	423.01
Totals	918	6342	5.424	343.96	1398	6612	5.363	354.61
	Senior-three-year-olds				Junior-four-year-olds			
180 or less	19	2216	5.246	116.26	18	2279	5.302	120.83
181 to 210	7	3277	5.358	175.57	13	4170	5.340	222.69
211 to 240	35	4565	5.201	237.43	44	4577	5.122	234.45
241 to 270	93	5418	5.326	288.57	113	5706	5.306	302.75
271 to 305	243	6374	5.390	343.59	292	6646	5.374	357.13
306 to 335	218	7503	5.388	404.24	262	7777	5.365	417.22
336 to 365	300	8142	5.516	449.11	341	8544	5.402	461.57
Totals	915	6946	5.427	376.98	1083	7233	5.369	388.35
	Senior-four-year-olds				Five-year-olds			
180 or less	19	2184	5.000	109.21	40	2422	5.186	125.60
181 to 210	12	4217	5.059	213.33	23	4078	5.161	210.48
211 to 240	26	4511	5.280	238.19	45	4778	5.171	247.09
241 to 270	63	5586	5.273	294.56	147	5922	5.319	314.97
271 to 305	206	6620	5.330	352.84	358	6952	5.281	367.11
306 to 335	196	7777	5.303	412.41	316	8111	5.316	431.19
336 to 365	260	9053	5.336	483.06	442	8994	5.367	482.74
Totals	782	7421	5.316	394.50	1371	7515	5.322	399.96

TABLE 4—(*Continued*)

Length (days)	No. of records	Aver. milk	Aver. fat per cent	Aver. fat	No. of records	Aver. milk	Aver. fat per cent	Aver. fat
	Six-year-olds				Seven-year-olds			
180 or less	28	2537	4.988	126.54	24	2608	4.991	130.17
181 to 210	21	3825	5.407	206.81	16	3942	5.248	206.88
211 to 240	36	4908	5.041	247.42	28	5040	5.077	255.86
241 to 270	104	6355	5.197	330.27	74	6111	5.124	313.11
271 to 305	270	6987	5.201	363.41	183	7129	5.200	370.71
306 to 335	230	8110	5.258	426.43	172	8149	5.156	420.20
336 to 365	364	9069	5.297	480.38	264	8983	5.267	473.09
Totals	1053	7637	5.250	400.95	761	7617	5.206	396.56
	Eight-year-olds				Nine-year-olds			
180 or less	20	2349	4.887	114.80	18	2011	4.777	96.06
181 to 210	13	3239	5.220	169.08	5	3723	5.017	186.80
211 to 240	20	4824	4.992	240.80	14	4913	5.292	260.00
241 to 270	50	5770	5.036	290.56	49	5591	4.960	277.29
271 to 305	127	6931	5.150	356.92	75	6987	5.235	365.76
306 to 335	98	8248	5.196	428.57	69	7813	5.106	398.93
336 to 365	205	8761	5.341	467.91	137	8445	5.287	446.47
Totals	533	7427	5.231	388.53	367	7133	5.196	370.61
	Ten-year-olds				Eleven-year-olds			
180 or less	11	1462	4.583	67.00	7	2132	4.818	102.71
181 to 210	2	4406	5.050	222.50	6	3245	4.859	157.67
211 to 240	7	4483	5.006	224.43	5	5079	4.733	240.40
241 to 270	25	5683	4.965	282.16	13	6595	5.013	330.62
271 to 305	60	7008	5.146	360.63	27	7176	5.225	374.93
306 to 335	50	7706	5.106	393.44	20	7537	5.084	383.20
336 to 365	77	8643	5.260	454.66	64	8615	5.239	451.31
Totals	232	7196	5.159	371.23	142	7334	5.169	379.13
	Twelve-year-olds and over				Summary (all ages)			
180 or less	17	2022	4.524	91.47	324	2197	5.051	110.97
181 to 210	6	3391	4.502	152.67	186	3652	5.265	192.28
211 to 240	7	5234	4.976	260.43	407	4517	5.170	233.51
241 to 270	17	5265	4.830	254.29	1203	5497	5.243	288.20
271 to 305	31	6153	5.024	309.19	3030	6376	5.299	337.88
306 to 335	30	7667	5.138	393.93	2788	7381	5.305	391.58
336 to 365	67	8102	5.073	410.99	4336	8138	5.372	437.19
Totals	175	6539	5.030	328.88	12274	6927	5.322	368.63

In examining the summary of this table, it is significant to observe that the average butterfat percentage of the cows that have completed lactation records in the Herd Improvement Registry of 336 to 365 days is almost identical with the previously published average of the breed based on cows that have completed yearly Register of Merit records. It is also significant to point out that the average production of the 4336 cows of all ages with complete lactation records of 336 to 365 days in length is 437.19 pounds of butterfat per cow, whereas the average yield per cow of 30,351 cows all ages that have completed yearly Register of Merit records from 1903 to 1937 inclusive, is 488.31 pounds of butterfat per cow. It would appear that there is not the extreme difference between Herd Improvement Registry lactation records and Register of Merit records as has often been assumed in the past. The grouping of the records by length of lactations, furnishes information regarding persistency of production. Turner (1) has stated that persistency of production tends to decline gradually with age. This fact seems to be substantiated by the summaries of the lactation records as presented in Table 5.

TABLE 5

Effect of age on length of individual lactation records

Length of lactations	Young cows: Yearlings, Jr. 2-year-olds, Sr. 2-year-olds, Jr. 3-year-olds, Sr. 3-year-olds, Jr. 4-year-olds, Sr. 4-year-olds		Mature cows: 5-year-olds, 6-year-olds, 7-year-olds, 8-year-olds		Old cows: 9-year-olds, 10-year-olds, 11-year-olds, 12-and-over	
	Number	Per cent	Number	Per cent	Number	Per cent
180 days or less	159	2.1	112	3.0	53	5.8
181 to 210 days	94	1.2	73	2.0	19	2.1
211 to 240 days	245	3.2	129	3.5	33	3.6
241 to 270 days	724	9.5	375	10.1	104	11.4
271 to 305 days	1899	24.9	938	25.2	193	21.1
306 to 335 days	1803	23.6	816	21.9	169	18.5
336 to 365 days	2716	35.6	1275	34.3	345	37.7
All lengths	7640		3718		916	

In examining this table, it will be seen that 84 per cent of the cows under five years of age showed complete lactation records of longer than 270 days. Of the cows from five to eight years of age, inclusive, 81 per cent showed complete lactation records of more than 270 days, whereas with the old cows past nine years of age, only 77 per cent had lactation records of more than nine months.

During the calendar year 1937, a total of 297 herds completed a year's work of herd testing. These herds were studied to learn several facts concerning herd management, such as culling, average age of cows in the

herds, the number and ages of first calf heifers and the production of these first calf heifers. It was found that at the beginning of the year's work of each herd, there were a total of 5864 cows in the herds that had previously freshened in their lifetimes. The average age of these 5864 cows at the beginning of the year's work was five years two months. During the year in these 297 herds, there were a total of 1582 new heifers that came into milk. It was interesting to observe the ages at which these heifers freshened and this is summarized in Table 6.

TABLE 6

Ages of heifers calving for first time during year

Age groups	Number	Age groups	Number	Age groups	Number
1–2	1	2–3	122	3–4	4
1–3	1	2–4	124	3–5	2
1–4	0	2–5	103	3–6	3
1–5	5	2–6	64	3–7	2
1–6	6	2–7	67	3–8	2
1–7	8	2–8	45	3–9	0
1–8	30	2–9	27	3–10	0
1–9	35	2–10	34	3–11	1
1–10	77	2–11	15	4–0	0
1–11	186	3–0	24	4–1	1
2–0	210	3–1	7	4–2	1
2–1	208	3–2	3		
2–2	161	3–3	3	Totals	1582

The average age at which these 1582 heifers calved was 26.49 months. Plum and Lush (2), reporting on a smaller number of animals in Iowa Cow Testing Associations, found the mean age for first calf Jersey heifers to be 25.5 months. Of course it is realized that many heifers required more than one service, and consequently the average age at first calving of 26.49 months is undoubtedly higher than what most Jersey breeders consider the ideal age for first freshening.

Breeders have often expressed the thought that the heifers calving during the year were a distinct handicap to the herd average. However, such is not the case. The herd average of any herd is based on the average number of cows in the herd and not on the average number of cows in milk. In most instances, the heifers calving for the first time during the year are milked until the year's work is completed and thus they have no dry period charged against them. On a calendar year basis, there were 723.55 first calf heifers in the herd and the average number of heifers in milk during the year was 701.37. The average butterfat yield of these first calf heifers, computed in the usual manner of figuring herd averages, is 391.16 pounds of butterfat. This is in excess of the average yield (370.69 lbs.) of all of the herds that have completed a year's work of herd testing in 1937. Consequently, it is evident that heifers calving for the first time during the year help the herd average rather than handicap it.

During 1937, there were purchased and added to these 297 herds, a total of 522 cows of milking age. The ages of these cows purchased are shown in Table 7.

TABLE 7

Ages of cows added to the herds during year

Age groups	Number of cows added	Per cent added
Yearlings	7	1.34
2-year-olds	79	15.13
3-year-olds	124	23.76
4-year-olds	103	19.73
5-year-olds	75	14.37
6-year-olds	55	10.54
7-year-olds	35	6.71
8-year-olds	13	2.49
9-year-olds	14	2.68
10-year-olds	8	1.53
11-year-olds	3	.58
12-and-over	6	1.15
Totals	522	

This table is of interest in illustrating the ages of cows which most breeders desire to purchase as additions to the herd. Likewise the ages of the cows culled or removed from the herds during the year is of equal interest. During the year a total of 1452 cows were removed from the 297 herds for one reason or another. In most instances, the supervisors do not report the cause of removal and hence it is not known whether these cows were culled because of low production, breeding difficulties or whether they were sold as surplus breeding stock. However, the ages of the cows that were culled during the year is of concern and this data has been summarized in Table 8. It will be noted from the information in this table that relatively few of the cows removed from the herds have been culled due to old

TABLE 8

Ages of cows removed from herds during year

Age groups of cows in herds	Number of cows in each age group	Number of cows sold or culled during year	Percentage of each age group sold or culled
Yearlings	413	43	10.41
2-year-olds	2321	313	13.49
3-year-olds	1395	238	17.06
4-year-olds	1102	202	18.33
5-year-olds	792	163	20.58
6-year-olds	620	145	23.39
7-year-olds	439	78	17.77
8-year-olds	323	91	28.17
9-year-olds	180	51	28.33
10-year-olds	152	47	30.92
11-year-olds	107	35	32.71
12 years and over	124	46	37.10
Totals	7968	1452	18.23

age, or died as a result of advanced age, for over 54 per cent of all the cows removed from all the herds during the year were under five years of age.

In compiling the foregoing data, it was noted that with Jersey herds on Herd Improvement Registry test, relatively few cows were used as nurse animals. Sixty-eight herds were on test in the northeastern section of the country and of this group only twenty-three used a total of thirty-eight cows as nurse animals during the year. There were 122 herds on test in the north central states and of this number thirty-four herds used a total of sixty-three nurse cows. There were sixty-three herds on test in the southern states and twenty-two of these herds used a total of sixty-seven nurse cows. Forty-four herds were on test in the Rocky Mountain and Pacific states and six of these herds used a total of just six nurse cows during the year. Apparently, only a small percentage of registered calves are raised with the use of nurse animals.

SUMMARY

It is difficult to summarize all of the data presented but such conclusions as can easily be drawn are as follows:

1. The percentage of dry cows in the herd during the year is one of the main factors affecting the herd average.
2. The average percentage of dry cows in all herds is approximately fifteen per cent.
3. Breeding efficiency and size of the herd are inversely related.
4. Culling can always be practiced with advantageous results regardless of the herd average.
5. Persistency of production tends to decline slightly with age.
6. Herd Test lactation records of ten months or more in length average between ten and twelve per cent less than Register of Merit records of similar length.
7. The average age at which Jersey heifers freshen is two years two and one-half months
8. The average age of all the cows in the herds that have freshened in their lifetimes is five years two months.
9. Heifers freshening for the first time during the year do not handicap the herd average.
10. More three- and four-year-old cows are added to the herds than cows of any other age.
11. Over 54 per cent of all the cows removed from the herds during the year were under five years of age.
12. Relatively few cows are used in registered herds as nurse animals.

REFERENCES

(1) TURNER, C. W. Persistency of fat secretion during the lactation period as affected by age. J. DAIRY SC. 10: no. 2. 1927.

(2) PLUM, M., AND LUSH, J. L. Freshening ages of purebred cows in Iowa Cow Testing Associations. J. DAIRY SC. 17: no. 9. 1934.

THE CHEMICAL COMPOSITION OF THE BLOOD OF THE DAIRY GOAT

O. B. HOUCHIN, W. R. GRAHAM, JR., V. E. PETERSON, AND C. W. TURNER
Department of Dairy Husbandry, University of Missouri[1]

The adaptability of the dairy goat for many types of experimental work is becoming recognized. For the study of the biochemistry of milk secretion and the influence of the hormones upon the growth of the udder and the stimulation of milk secretion, the small size yet easily obtainable milk yield of the dairy goat makes it a most suitable animal for preliminary experiments.

In the use of the dairy goat for experimental purposes it is desirable to establish norms or standards with which the experimental conditions may be compared with studies of the precursors of milk.

Fish and Hayden (1) and Hayden and Fish (2) reported values for certain constituents of the blood of the normal and castrated male goat. For the purpose of studying carbohydrate metabolism, Cutler (3) gave figures on the glucose and inorganic phosphorus content in the blood of normal goats and during various pathological conditions. At about the same time Lintzel (4) published material concerning milk precursors which included data on the blood of lactating goats. Included in his data are the figures for both arterial and mammary venous blood.

In this communication average values for the more important constituents of arterial and venous blood of dry and lactating dairy goats are reported as tentative normal standards.

The data to be presented were obtained in the course of a series of experiments having as their objective the determination of certain precursors of milk (5, 55, 59, 63).

The blood used for analysis was taken from the carotid artery, which is recognized as typical arterial blood, and from the mammary vein. Graham, Turner and Gomez (6) described a method of preparing the artery by exteriorization for the purpose of obtaining arterial blood. The mammary venous blood was taken to represent typical blood from the udder since all peripheral veins were previously ligated. Precautions followed in drawing the blood samples were given in the publication of Graham, Houchin and Turner (5).

The analytical methods used were applied to both whole blood and plasma. The blood was prevented from clotting by the use of potassium oxalate. Plasma and cells were separated by centrifugation. Where the

Received for publication October 25, 1938.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 580.

procedures necessitated the use of filtrates, *i.e.*, N–P–N and amino acid, sufficient quantities were prepared to take care of all determinations. The analyses were run until duplicates checked within one per cent.

Total nitrogen was determined by the Kjeldahl method. Urea was determined on whole blood and plasma by the urease, manometric method of Van Slyke (7); in case sufficient blood was not available for the former metho , the determination was made on the Folin-Wu filtrate by the method of Van Slyke (8). Amino nitrogen was determined by the Danielson (9) method.

Amide nitrogen was determined by digesting the Folin-Wu precipitate to break off the amide particle according to the method described by Bliss (10), aerated, then nitrogen determined manometrically using sodium hypobromite. Non-protein nitrogen was determined on the Folin-Wu filtrate by means of the manometric micro-Kjeldahl method described by Van Slyke (11).

The fibrin of the plasma was diluted with isotonic NaCl solution and precipitated with calcium chloride, Howe (12). Micro-Kjeldahl digestion and hypobromite manometric determination were then made for nitrogen (11).

Albumin nitrogen plus non-protein nitrogen in plasma was fractionated from the globulin by Howe's method (12) then nitrogen determined by the Kjeldahl method. The globulin plus fibrin fraction was calculated by subtracting the concentration of albumin plus non-protein nitrogen from the total plasma nitrogen. To find the albumin nitrogen, the value obtained for non-protein nitrogen was subtracted from the albumin plus non-protein nitrogen fraction. The fibrin value was subtracted from the globulin plus fibrin fraction in calculating the globulin nitrogen content.

Blood glucose, without other reducing materials, was determined by use of the Shaffer, Hartman and Symogyi (13, 14) iodometric titration method. In a majority of cases the colorimetric method of Mendel and Goldscheider (15) was used to determine lactic acid. To verify results by the above method, some determinations were made by the von Furth-Charness method (16) as modified by West (17).

The simultaneous manometric method of Van Slyke and Neill (18) was used for oxygen and carbon dioxide, oxalate was used as anticoagulant and sodium floride to prevent glycolysis and bacterial action. The hemoglobin content was found by the method of Newcomer (19) and cell volume by the use of hematocrit tubes.

Both the inorganic and organic phosphates were determined by the method of Whitehorn (20) and Benedict (21).

The data on the composition of the whole blood and plasma of both lactating and non-lactating goats were combined (Table 1). The samples from lactating animals were taken at increasing intervals after milking. Only a few of the samples were analyzed for all of the above mentioned constituents. In addition to the average value, the variation in each constituent is

TABLE 1

Chemical composition of goat blood

Constituents	Arterial				Venous			
	No. of samples	Ave. conc.	Range		No. of samples	Ave. conc.	Range	
			High	Low			High	Low
Whole blood								
Total nitrogen (mg. %)	32	2505.04	2947.30	2142.70	32	2507.00	2850.50	2133.90
Urea nitrogen (mg. %)	32	22.31	33.88	14.53	32	23.16	36.72	14.80
Amino nitrogen (mg. %)	24	9.06	11.83	7.19	23	8.37	10.45	6.28
Amide nitrogen (mg. %)	6	128.40	152.70	119.57	6	119.31	141.40	103.53
Non-protein nitrogen (mg. %)	19	48.52	63.20	35.90	19	48.50	75.90	34.67
Sugar (mg. %)	28	59.07	77.37	33.93	27	48.12	72.35	34.88
Lactic acid (mg. %)	28	53.88	134.30	21.29	31	50.54	100.00	23.56
Total phosphorus (mg. %)	19	22.72	33.48	13.37	19	23.21	32.48	18.02
Inorganic phosphorus (mg. %)	15	7.69	11.91	5.76	14	6.91	8.72	5.63
Oxygen (vol. %)	25	12.80	15.41	9.56	25	8.48	12.60	4.62
Carbon dioxide (vol. %)	25	46.74	56.91	36.65	25	53.31	62.02	43.92
Hemoglobin (gm. %)	16	9.26	14.40	5.94	15	9.41	12.00	8.02
Cell (vol. %)	7	28.84	36.30	24.09	7	29.21	38.60	24.56
Plasma (gm. %)								
Total nitrogen	26	1228.20	1708.80	1043.70	26	1204.45	1518.70	1090.00
Alb. + NPN	23	468.98	612.80	298.90	24	472.84	637.60	277.60
Globulin + fibrin	23	759.22	1145.10	597.10	24	731.61	933.70	544.40
Urea nitrogen	4	20.23	26.86	17.16	4	19.69	26.98	16.90
Amino nitrogen	6	7.70	9.82	6.06	6	7.19	9.27	5.63
Amide nitrogen	5	96.34	103.20	81.24	8	69.39	98.35	41.64
Non-protein nitrogen	6	38.33	43.20	32.66	7	39.28	47.00	28.82
Fibrin nitrogen	6	83.48	113.03	66.38	6	100.88	124.90	81.17

TABLE 2

The relation of the blood from the lactating and non-lactating goat

Constituents	Arterial				Venous			
	Lactating		Non-lactating		Lactating		Non-lactating	
	No. of samples	Ave. conc.	No. of samples	Ave. conc.	No. of samples	Ave. conc.	No. of samples	Ave. conc.
Whole blood								
Total nitrogen (mg. %)	23	2511.10	9	2490.00	23	2540.60	9	2421.20
Urea nitrogen (mg. %)	22	23.07	10	21.76	22	23.64	10	22.12
Amino nitrogen (mg. %)	16	9.12	8	8.93	15	8.47	8	8.17
Amide nitrogen (mg. %)	3	135.23	3	121.50	3	122.40	3	116.20
Non-protein nitrogen (mg. %)	11	51.21	8	44.76	10	48.99	9	47.97
Sugar (mg. %)	20	59.87	8	57.08	20	45.27	7	56.28
Lactic acid (mg. %)	20	58.77	6	37.35	21	50.08	9	43.61
Total phosphorus (mg. %)	17	23.55	2	20.63	16	23.60	2	20.85
Inorganic phosphorus (mg. %)	15	7.69	.....	.....	13	6.91	.....	.....
Oxygen (vol. %)	22	12.64	3	14.15	22	7.24	3	9.14
CO_2 (vol. %)	22	47.32	3	42.45	22	53.89	3	49.00
Hemoglobin (gm. %)	9	8.73	7	9.90	10	9.34	5	9.27
Cell (vol. %)	5	28.30	2	30.20	5	27.89	2	31.68
Plasma (mg. %)								
Total nitrogen	12	1236.60	14	1220.90	13	1193.20	13	1215.50
Alb. + NPN	11	478.00	11	453.30	12	459.10	12	486.80
Globulin + fibrin	11	758.60	11	767.60	12	734.10	12	729.00
Urea nitrogen	2	22.80	2	17.68	2	21.94	2	17.43
Amino nitrogen	3	8.30	3	7.08	3	7.90	3	6.45
Amide nitrogen	3	100.50	3	82.40	4	80.10	4	57.70
Non-protein nitrogen	2	40.17	4	37.40	3	42.06	4	36.70
Fibrin	5	86.31	1	69.33	4	103.97	2	94.70

indicated by the presentation of the range. Most of the samples came from non-pregnant goats during their lactating and subsequent dry period.

Comparison of the arterial and venous (mammary) blood show only a few significant differences, *i.e.*, amide nitrogen, sugar, lactic acid, oxygen, carbon dioxide and fibrin. The ranges of both are usually comparable. Some constituents showed higher arterial blood values than the corresponding venous values although the averages may be similar. As should be expected the range levels of the oxygen are much higher in the arterial blood than in venous blood. On the other hand, the carbon dioxide in the arterial blood is low and in the venous blood high.

The range for total nitrogen showed the greatest absolute variation. However, the per cent variation of the lactic acid values was greater than that of any other constituent. Many of the constituents have range differences that show no large variation.

A majority of the experiments were on lactating goats. At the latter part of the experiment some determinations were made on dry animals. Only averages were presented to show the relation between the lactating and non-lactating goats (Table 2). Some constituents show considerable difference between the lactating and non-lactating condition. The oxygen content was found to be higher in the non-lactating animals, while the carbon

TABLE 3

Relation of goat blood to other domestic animals

	Total plasma N	Urea N	NPN	Amino N	Sugar	Inorganic phosphorus	Hemoglobin	Cell
	mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*gm. %*	*vol. %*
Cattle	1177.6 (2)	12.2 (2)	30.0 (27)	7.19 (36)	53.0 (39)	5.7 (2)	10.90 (52)	34.3 (36)
Horse	1296.0 (23)	18.7 (21)	25.8 (31)		109.0 (31)			33.0 (51)
Sheep	1054.4 (22)	13.0 (26)	28.5 (33)	4.76 (37)	63.90 (40)	7.1 (44)		
Dog	1131.2 (22)	11.7 (28)	30.8 (28)	6.70 (28)	82.00 (28)	3.5 (45)	14.11 (47)	47.7 (47)
Rabbit	1072.0 (23)	13.0 (26)	31.0 (32)		124.0 (43)	4.5 (46)		
Swine	1216.0 (22)	17.3 (29)	31.4 (34)		128.0 (34)		11.95 (48)	47.8 (48)
Rat	1040 (23)	15.6 (29)	42.0 (35)		122 (42)			48.0 (40)
Hen		4.53 (25)	38.5 (25)		212 (25)	3.96 (2)		
Cat	1347.2 (22)	30.3 (26)	52.6 (32)					
Man	1120 (24)	17.1 (30)	35.6 (30)	6.40 (38)	112.0 (30)			45.6 (50)
Goat*	1128	22.31	48.52	9.60	59.1	7.70	9.26	28.8

Numbers in parenthesis denote reference from which taken.
* Data from this paper.

dioxide value was lower and showed a lower R. Q. Lactic acid was reversed in regard to A–V difference in the lactating and non-lactating animal, being positive in those lactating. The venous blood of the lactating goat had less glucose than that of the non-lactating goat, while the arterial values were virtually the same. Values for other constituents did not show much variation between the lactating and non-lactating condition.

Goat's blood has been found to be relatively similar to the blood of other domestic animals in regard to the concentration of its constituents (Table 3). A tabulation of the composition of the blood of a number of domestic and laboratory animals has been prepared for comparison with average goat blood as presented in Table 1.

For most constituents the value for the goat is intermediate in comparison with other animals. The cell volume, however, is considerably lower than that of most animals included. As would be expected the hemoglobin value for the goat is lower than that of other animals but not to as large extent as the cell volume. In a few cases such as amino acid and inorganic phosphorus, the goat blood shows a higher value than any other animal. From these data it would seem that goat blood is not a peculiar type but is in most respects similar in composition to that of other species reported.

DISCUSSION

During the past few years, this department has been engaged in a study of the precursors of milk using dairy goats as experimental animals. This work was initiated following the development by Herrick and Baldes (53) of a technique for the measurement of the blood flow in normal intact animals. If this method could be adapted to the measurement of the arterial or venous blood passing through the mammary gland, the milk precursors could be studied quantitatively and the efficiency of the udder as a converter could be determined.

The classical experiments of Kaufman and Magne (54) which have been confirmed abundantly indicate that considerable quantities of glucose are removed from the blood during its passage through the mammary gland. These observations have led to the opinion that glucose is the precursor of lactose. However, when the blood flow measurements were combined with the analyses of arterial and venous glucose, it was observed that considerably more lactose was being secreted in the milk than could be accounted for by the removal of glucose from the blood, Graham (55). It appeared necessary to examine the blood for other carbohydrate-forming compounds which might be taken up by the udder. It was subsequently observed that considerable amounts of lactic acid were also removed from the blood in its passage through the gland. In further experiments it was calculated that 85 per cent of the lactose formed during the period could be accounted for theoretically from the glucose and lactic acid removed from the blood. It is

interesting to note in passing that considerable quantities of lactic acid have since been observed by Shaw and Petersen (56) to be withdrawn from the blood of the dairy cow during its passage through the lactating udder. In further confirmation of the above evidence that lactic acid is a precursor of lactose, Petersen and Shaw (57) showed that lactose could be synthesized *in vitro* from a substrate containing glucose and lactic acid to which macerated mammary gland tissue from lactating cows was added.

Since the experiments of Cary (58) and others showed that amino acids are removed from the blood during its passage through the lactating mammary gland, it has been generally assumed that the amino acids were the chief, if not the sole precursors of milk protein. Our recent studies have shown quite definitely that insufficient amino acid is taken up by the active gland to account for more than a small part of the milk protein secreted (59). Shaw and Petersen (60) have recently confirmed this observation and have claimed further that not more than 35 per cent of the milk proteins could be formed from this source (61). Further light was thrown on the nitrogen metabolism of the lactating mammary gland by our observation that urea was being produced in amounts equivalent to the nitrogen contained in the amino acids taken up by the udder. Recently the presence of arginase, an enzyme capable of synthesizing urea, was observed to be present in the lactating but not in the non-lactating mammary gland (62).

From these observations it would appear that the possibility exists that certain amino acids may be deaminized and converted into lactic acid which would be a further source of carbohydrates. Consequently, the precursors of milk protein must be sought in other nitrogenous compounds of the blood. Further study revealed that in addition to amino N, globulin and unknown fractions of the non-protein nitrogen of the blood were being taken up by the active mammary gland (50). Work is now in progress having as its object the determination of the particular fraction of the nitrogen partition of the blood utilized by the mammary gland.

In the course of the investigations discussed above, a number of the constituents of goat's blood were determined. As the results were presented chiefly as arterial-venous differences, it seemed desirable to bring together the average values for arterial and venous goat blood obtained from this work. It is hoped that these values will be useful in the development of normal standards of dairy goat blood.

SUMMARY AND CONCLUSIONS

Data were presented on the range and average composition of certain constituents of the whole blood and plasma of dairy goats. A comparison of the arterial and venous blood of lactating and non-lactating animals was made.

For purposes of comparison, a tabulation of comparative data on the

composition of the blood of other species was presented. No significant differences in the goat plasma were noted in comparison with other mammals. It will be seen that the cell volume of the goat is unusually low probably due to the small size of the red blood corpuscle.

REFERENCES

(1) FISH, P. A., AND HAYDEN, C. E. A comparison of the blood of a normal and two castrated billy goats. Report of N. Y. State Vet. College, No. 28: 76. 1927.

(2) HAYDEN, C. E., AND FISH, P. A. The normal blood of some domesticated animals. Report of N. Y. State Vet. College, No. 18: 207. 1929.

(3) CUTLER, J. I. Blood sugar of the goat. J. Biol. Chem. 106: 653. 1934.

(4) LINTZEL, W. Untersuchnagen uber den Chemismus der Milchfettbildung von Tutternung. Zeit. Zucht., Reihe, B. 29: 219. 1934.

(5) GRAHAM, JR., W. R., HOUCHIN, O. B., AND TURNER, C. W. The production of urea in the mammary gland. J. Biol. Chem. 120: 29. 1937.

(6) GRAHAM, JR., W. R., TURNER, C. W., AND GOMEZ, E. T. A method for obtaining arterial blood from the goat. Missouri Agr. Exp. Sta. Res. Bul. 260. 1937.

(7) VAN SLYKE, D. D. Determination of urea by gasometric measurement of the carbon dioxide formed by the action of urease. J. Biol. Chem. 73: 695. 1927.

(8) VAN SLYKE, D. D. The manometric determination of urea in blood and urine by the hypobromite reaction. J. Biol. Chem. 83: 449. 1929.

(9) DANIELSON, I. S. Amino acid nitrogen in blood and its determination. J. Biol. Chem. 101: 505. 1933.

(10) BLISS, S. The amide nitrogen of blood. II. A quantitative method. J. Biol. Chem. 81: 129. 1929.

(11) VAN SLYKE, D. D. Gasometric micro-Kjeldahl determination of nitrogen. J. Biol. Chem. 71: 235. 1927.

(12) HOWE, P. E. The determination of proteins in blood—a micro-method. J. Biol. Chem. 49: 109. 1921.

(13) SHAFFER, P. A., AND HARTMAN, A. F. II. Methods for the determination of reducing sugars in blood, urine, milk and other solutions. J. Biol. Chem. 45: 365. 1921.

(14) SHAFFER, P. A., AND SOMOGYI, M. Copper-iodometric reagents for sugar determinations. J. Biol. Chem. 100: 695. 1933.

(15) MENDEL, B., AND GOLDSCHEIDER, I. Eine kolorimetrische Mikromethode zur quantitativen Bestimmung der Milchsaure im Blut. Biochem. Zschr. 164: 163. 1925.

(16) FURTH, O. VON, AND CHARNESS, D. Uber die quantitative Bestimmung der daraus abspaltbaren aldehydmerge. Biochem. Zschr. 26: 199. 1910.

(17) WEST, E. S. An improved lactic acid apparatus. J. Biol. Chem. 92: 483. 1931.

(18) VAN SLYKE, D. D., AND NEILL, J. M. The determination of bases in blood and other solutions by vacuum extraction and manometric measurement. J. Biol. Chem. 61: 523. 1924.

(19) NEWCOMER, H. S. A new optical instrument for the determination of hemoglobin. J. Biol. Chem. 55: 569. 1923.

(20) WHITEHORN, J. C. A method for the determination of lipoid phosphorus in blood and in plasma. J. Biol. Chem. 62: 133. 1924–25.

(21) BENEDICT, S. R., AND THEIS, R. C. A modification of the molybdic method for the determination of inorganic phosphorus in serum. J. Biol. Chem. 61: 63. 1924.

(22) WOOLSEY, J. H. Studies in the blood relationship of animals as displayed in the composition of the serum proteins. II. J. Biol. Chem. 14: 433. 1913.

(23) ROBERTSON, T. B. Principles of biochemistry, 2nd edition, p. 310. 1924.
(24) PETERS AND VAN SLYKE. Quant. Clin. Chem. Interp. Baltimore, 1932.
(25) HELLER, V. G., AND PURSELL, L. Chemical composition of the hen during its life cycle. J. Biol. Chem. 118: 549. 1937.
(26) FOLIN, O., AND MORRIS, J. L. The normal protein metabolism of the rat. J. Biol. Chem. 14: 509. 1913.
(27) ANDERSON, A. K., HONEYWELL, H. E., AND SANTZ, A. C. The composition of normal rat blood. J. Biol. Chem. 86: 157. 1931.
(28) HADEN, R. S., AND ORR, T. G. Chemical findings in the blood of the normal dog. J. Exp. Med. 37: 365. 1923.
(29) RODERICK, L. M., AND SCHALK, A. F. Collected studies on hog cholera. North Dakota Agr. Exp. Sta. Bul. 210: 3. 1927.
(30) HAMMETT, F. S. Studies of variation in the chemical composition of human blood. J. Biol. Chem. 41: 599. 1920.
(31) HOLT, R. L., AND REYNOLDS, F. A. K. Blood chemistry of the horse. J. Am. Vet. M. Ass. 65: 732. 1924.
(32) FOLIN, O., AND DENIS, W. J. Protein metabolism from the standpoint of blood and tissue analysis. VI. On uric acid, urea and total NPN in human blood. J. Biol. Chem. 20: 629. 1915.
(33) SCHEUNERT, A., AND PELCHRZIM, V. Blutzuckerkonzentration ber Kulen unter Erafluzoz der Lactationsentensitat. Biochem. Zeit. 139: 17. 1923.
(34) HEWITT, E. A. The blood sugar level of the bovine. J. Am. Vet. M. Ass. 77: 362. 1930.
(35) CHANUTIN, A., AND SILVETTE, H. The influence of fasting and creatine feeding upon the creatine content of the tissues and blood of the white rat. J. Biol. Chem. 80: 589. 1928.
(36) BLACKWOOD, J. H. The absorption of milk precursors by the mammary gland. The relation of amino acid absorption to protein synthesis. Biochem. J. 26: 772. 1932.
(37) BLAU, N. F. The total free amino acid nitrogen in the blood. J. Biol. Chem. 56: 861. 1923.
(38) FOLIN, I., AND BERGLUND, H. Retention and distribution of amino acids with special reference to urea formation. J. Biol. Chem. 51: 395. 1922.
(39) HODGSON, R. E., RIDDELL, W. H., AND HUGHES, S. Factors influencing the blood-sugar level of dairy cattle. J. Agr. Res. 44: 357. 1932.
(40) TRAUTMAN, A., LUY, P., AND SCHMITT, J. Chemical and physicochemical studies on the blood of healthy sheep. Biochem. Zschr. 241: 260. 1931.
(41) BLACKWOOD, J. H., AND STIRLING, J. D. The true sugar content in the blood of cattle. Biochem. J. 26: 1127. 1932.
(42) HAUBETZ, M. C. Liver glycogen, with a note on blood sugar level. Am. J. Physiol. 114: 551; L. B. DOTTI, Am. J. Physiol. 114: 538. 1934.
(43) WATKINS, O., AND SMITH, G. V. Biochemical studies on effect of adrenalin upon nitrogen metabolism of rabbits. Am. J. Physiol. 96: 28. 1931.
(44) TRAUTMAN, A., AND SCHMITT, J. Chemical studies on the blood of sheep. Biochem. Zschr. 241: 270. 1931.
(45) ABDERHOLDEN, E. Zur quantitative verglenchender Analyse des Blutes. Z. Physiol. Chem. 25: 65. 1898.
(46) BROWN, W. H. Calcium and inorganic phosphorus in blood of rabbits; results of repeated and prolonged observations on normal rabbits. J. Exp. M. 47: 539. 1928.
(47) TIRCHSENRING, *et al.* Blood regeneration studies. 2. Observations on the blood of

normal dogs with special reference to the measurement of volume, erythrocytes, leucocytes and nitrogen constituents. Am. J. Physiol. 199: 391. 1932.

(48) OGELSBY, W. T., HEWITT, E. A., AND BERGMAN, H. G. Certain chemical and morphological phases of the blood of normal and cholera infected swine. Iowa State College J. of Sci. 6: 227. 1932.

(49) SWANSON, P. P., AND SMITH, A. H. Inorganic salt in nutrition. IV. Changes induced in the blood by a ration deficient in inorganic constituents. J. Biol. Chem. 98: 479. 1932.

(50) PEARL, A., AND MINER, J. R. Biometric study of relative cell volume of human blood in normal and tuberculosis males. Bul. Johns Hopkins Hosp. 40: 3. 1927.

(51) NESER, C. L. LXXXI. The blood of equines. Biochem. J. 16: 770. 1922.

(52) MCCAY, C. M. The hemoglobin and total phosphorus in the blood of cows and bulls. J. DAIRY SC. 14: 373. 1931.

(53) HERRICK, J. F., AND BALDES, E. J. The thermo-stromuhr method of measuring blood flow. Physics 1: 407. 1931.

(54) KAUFMAN, M., AND MAGNE, H. Sur la consommation der glucose du savy par le tissu de les glande mammsire. Compt. rend. 143: 779. 1906.

(55) GRAHAM, JR., W. R. The utilization of lactic acid by the lactating mammary gland. J. Biol. Chem. 122: 1. 1937.

(56) SHAW, J. C., BOYD, W. L., AND PETERSEN, W. E. Blood glucose and lactic acid in relation to milk secretion. Proc. Soc. Exp. Biol. 38: 579. 1938.

(57) PETERSEN, W. E., AND SHAW, J. C. In vitro synthesis of lactose. Science 86: 398. 1937.

(58) CARY, C. A. Amino acids of the blood as the precursors of milk proteins. J. Biol. Chem. 43: 477. 1920.

(59) GRAHAM, JR., W. R., PETERSON, V. E., HOUCHIN, O. B., AND TURNER, C. W. The utilization of fractions of the nitrogen partition of the blood by the active mammary gland. J. Biol. Chem. 122: 275. 1938.

(60) SHAW, J. C., AND PETERSEN, W. E. The ratio of arterio-venous differences of certain substance to quantities secreted by the mammary gland. Proc. Am. Physiol. Soc. 50th Ann. Meeting, 183. 1938.

(61) SHAW, J. C., AND PETERSEN, W. E. Amino acids and other non-protein nitrogen blood substance in relation to milk secretion. Proc. Soc. Exp. Biol. 38: 632. 1938.

(62) SHAW, J. C., AND PETERSEN, W. E. Arginase in the mammary gland. Proc. Soc. Exp. Biol. 38: 631. 1938.

(63) GRAHAM, JR., W. R., HOUCHIN, O. B., PETERSON, V. E., AND TURNER, C. W. The efficiency of the mammary gland in the production of milk. Am. J. Physiol. 122: 150. 1938.

STUDIES ON THE COMPOSITION OF BOVINE BLOOD AS INFLUENCED BY GESTATION, LACTATION, AND AGE[1]

WADARAN L. KENNEDY, ARTHUR K. ANDERSON, S. I. BECHDEL, AND J. F. SHIGLEY

From the Departments of Dairy Husbandry and Agricultural and Biological Chemistry of The Pennsylvania State College, State College, Pennsylvania

In 1930 Anderson, Gayley, and Pratt (1), of this laboratory, published a paper on the chemical composition of bovine blood. In their work blood samples were taken at random from the dairy herd, the object being to determine normal values for many of the blood constituents. Perhaps the most interesting point brought out in their study was the wide variation in the glucose content of the blood of animals in the different age groups.

The work reported in this paper is a more detailed study of the effect of gestation and lactation on a few of the more important constituents of the blood which might be expected to vary under these conditions. There is also reported a detailed study of blood glucose values in calves' blood in an attempt to explain the high sugar values found as compared with the blood of older animals.

METHODS

Blood samples were taken between 6:00 and 7:00 A.M., usually before feeding. Two samples were drawn from the jugular vein, one in 20 mg. of potassium oxalate per 10 cc. of blood and the other without oxalate. The oxalated sample was used for the determination of non-protein nitrogen and glucose. From the sample without oxalate the serum was separated by means of a centrifuge within one hour after drawing and the serum used for the determination of calcium and phosphorus.

Non-protein nitrogen was determined by the method of Folin and Wu (7), and glucose by the method of Folin (6), on a laked blood filtrate. In most cases the glucose method was modified by using a standard containing 0.1 mg. of glucose per 2 cc. This was necessary because the glucose content of cow's blood is about one-half that of human blood.

Serum was analyzed for calcium by the method of Clark and Collip (4), and for phosphorus by the method of Youngburg (24).

In the studies on carbohydrate tolerance the method of Killian (13) was

Received for publication September 29, 1938.

[1] Paper No. 847 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This paper is part of a thesis presented by Wadaran L. Kennedy to the faculty of the Graduate School of The Pennsylvania State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

used. A technical grade of glucose was dissolved in water to make a 50 per cent solution and administered as a drench.

EXPERIMENTAL

1. *Influence of Gestation and Lactation on the Composition of Bovine Blood*

Since the complete analysis of blood requires much time, it was felt that the work might be lightened by a careful selection of determinations to be made. Many of the determinations made in a blood analysis are for nitrogenous constituents which are included in the non-protein nitrogen fraction. Therefore it was felt that non-protein nitrogen values would indicate whether or not any of the components of this fraction were varying. A knowledge of non-protein nitrogen values would also serve as an excellent measure of kidney function throughout the experimental period. Since previous work (1) had indicated that the glucose content of bovine blood varied considerably with age, it was felt that this determination should be included. Calcium and phosphorus determinations were included because during pregnancy and lactation extraordinary demands are placed on the reserves of these elements in the body.

In this study the blood of the experimental animals was analyzed at approximately weekly intervals from the time of breeding through one year of lactation. Since the time required to follow the same group of animals throughout the entire period of gestation and lactation was prohibitive, two groups of animals were used. Group I consisted of five heifers (two Holsteins, two Guernseys, and one Jersey) that had been bred for the first time and Group II consisted of five cows (four Holsteins and one Jersey) that had just dropped their first calf. The animals were selected from the purebred dairy herd of The Pennsylvania State College. They received the regular ration fed the dairy herd. All samples were analyzed in duplicate and the average of these results was used.

The data obtained in this study indicated that gestation and lactation had little effect on the blood constituents determined. Since the presentation of these data would require a table containing over 1,300 values, it appeared desirable to summarize the data in Table 1 and comment on a few of the results that perhaps are significant.

Non-protein nitrogen.—Non-protein nitrogen values ranged from 21.01 to 45.71 mg. per 100 cc. of blood, the average value being 32.82. These values do not differ significantly from those which one would expect to find for normal human blood. The average value during gestation is about 2.0 mg. less than the average value during lactation. The results agree with those of Anderson, Gayley and Pratt (1) and Hayden and Sholl (10).

Immediately after parturition non-protein nitrogen values were above

TABLE 1

Showing the composition of cow's blood from the time of breeding through one year of lactation

Number of animals	Constituents determined	Number of determinations	High	Low	Average
	From breeding to parturition				
				mg. per 100 cc.	
5	Non-protein nitrogen	164	45.71	21.01	31.83
	Glucose	171	68.00	60.70	52.51
	Calcium	166	12.90	7.70	10.73
	Phosphorus	161	7.54	4.16	5.64
	From parturition through lactation				
5	Non-protein nitrogen	168	45.58	21.24	33.82
	Glucose	171	52.33	37.80	51.73
	Calcium	166	13.20	9.00	11.08
	Phosphorus	164	7.49	3.38	5.23
	From breeding through lactation				
10	Non-protein nitrogen	332	45.71	21.01	32.82
	Glucose	342	68.00	37.80	52.12
	Calcium	332	13.20	7.70	10.91
	Phosphorus	325	7.54	3.38	5.44

average, the values of the five animals being 34.28, 39.09, 41.73, 43.33 and 44.11 mg.

During a period of severe cold weather, where the temperature remained below zero most of the time for about three weeks, non-protein nitrogen values were from 5 to 10 mg. higher than they were just previous and just after this period. Since the animals did not drink as much water as usual during the cold weather, this may explain the high results. Peters and Van Slyke (16) state that non-protein nitrogen values in human blood are increased with a low water intake.

Glucose.—The results for glucose gave an average of 52.12 mg. per 100 cc. of blood for 342 determinations. Values ranged from 31.8 to 71.4 mg. The low value is questionable, since for the previous week this animal gave a value of 46.6 mg. and on the following week 50.7 mg. At no other time throughout the experiment did the value for this animal go below 46.2 mg. The second lowest value for any animal was 37.8 mg. The average value of 52.12 mg. is in close agreement with that reported by Hodgson, Riddell and Hughes (12); Anderson, Gayley and Pratt (1); and Hayden and Sholl (10).

There was no significant difference in the glucose content of the blood of the pregnant non-lactating animals and of the lactating group. The former group gave an average of 52.51 mg. while the latter gave 51.73 mg. Considerable work has been reported on the effect of lactation on blood sugar values. Among those who have found low values for lactating animals may

be mentioned Widmark and Carlens (23); Hewitt (11); Hodgson, Riddell and Hughes (12); Schlotthauer (19); Schwarz (20); Schwarz and Mezler-Andelberg (21). Moussu and Moussu (15) and Fish (5) report higher values for lactating cows than for dry cows. Scheunert and Pelchrzim (18); Awdejewa, Prowatorowa, Sawitsch, and Thal (3); and Hayden and Fish (9) found no difference in the blood sugar level between dry and lactating cows.

Calcium.—The values for calcium on blood serum were fairly constant throughout the experiment, most of the values falling between 9.0 and 11.5 mg. per 100 cc. As a result of 332 determinations, values were obtained ranging from 7.7 to 13.2 mg., the average value being 10.91 mg. per 100 cc. of serum. Pregnancy and lactation apparently had little effect on the calcium content of the blood, the average value for the former period being 10.73 mg. and for the latter 11.08 mg. Our results agree with the findings of Meigs, Blatherwick and Cary (14); Robinson and Huffman (17); and Haag (8).

Phosphorus.—The values for inorganic phosphorus in the serum remained fairly constant throughout pregnancy and lactation. As a result of 325 analyses, values were obtained ranging from 3.38 to 7.54 mg. per 100 cc. The average value was 5.44 mg. per 100 cc. During pregnancy the average value was 5.64 mg., while during lactation it was 5.23 mg. In previous work from this laboratory (1) it has been shown that serum phosphorus values decrease with age. Since in this experiment we were dealing with young animals bearing their first calf, it is quite possible that the slightly lower values for phosphorus during lactation are due to an age factor rather than to lactation. The above values agree closely with the results obtained by others (1, 14, 17).

2. *Blood Sugar Studies*

If one compares the composition of human blood with that of the cow, the most striking difference is found in the sugar content. Normal human blood contains in the neighborhood of 100 mg. of glucose per 100 cc. whereas cow's blood contains approximately 50 mg. per 100 cc. When the glucose value of human blood reaches 45 mg. per 100 cc. it is said to be at the convulsive level. Why a cow can be normal with the blood sugar at the convulsive level for humans is an interesting question. Since sheep (22) and goats (9) also have low blood sugar values it would appear that a low level of blood sugar is characteristic of ruminants. Dogs and horses (9) have blood sugar values approximating that of man, while chickens (9) and rats (2) have definitely higher values than man. From studies with the use of insulin in diabetes it is known that there is a definite relationship between the amount of insulin given to an individual and his blood sugar level. It would be an interesting study to determine the insulin content of the pan-

creases of different species and to observe any correlation between these findings and the normal blood sugar levels for these species.

Blood sugar and age.—A very interesting fact brought out in a previous study (1) of the blood sugar of bovines was that in young calves blood sugar values are twice as high as in mature cows. This has suggested a more detailed study of the influence of age on blood sugar values. For this study two groups of calves were selected; the first group consisting of three calves less than a week old and the second group of two calves five months of age. The two groups were selected in order to cover approximately the first year of life in the six months available for the work. Samples were taken usually at 8:30 A.M. after feeding and at weekly intervals covering the first 47 weeks of life. The results are shown graphically in Figure 1.

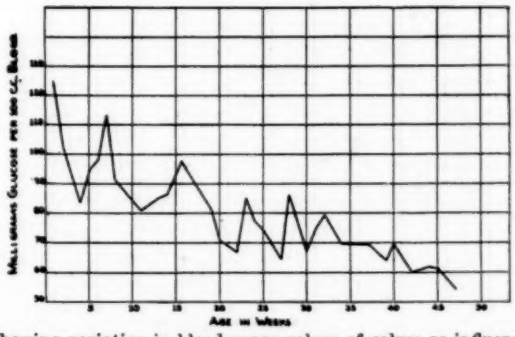

FIG. 1. Showing variation in blood sugar values of calves as influenced by age.

It may be observed from Figure 1 that for the first week of life the average blood sugar value was 125.0 mg. per 100 cc. of blood. By the end of the second week the average value had dropped to 102.5 mg. and by the end of the fourth week to 83.3 mg. For the next three weeks values increased and then they decreased. Considerable variation was observed until the thirtieth week when the curve became quite smooth to the end of the period when a value of 54.4 mg. was reached, which is about the value to be expected for a mature animal.

Diurnal variation in blood sugar.—The fluctuations in the first half of the curve shown in Figure 1 suggested a possible diurnal variation in blood sugar values. In order to study this an experiment was run on two calves over a period of 24 hours. Blood samples were taken frequently throughout this period. The results are shown graphically in Figure 2. The first samples were taken before feeding in the morning and the last samples before feeding the following morning. The blood sugar averaged 80.8 mg. per 100 cc. on the first morning and 80.5 mg. on the second. Samples taken one hour after the morning feeding showed an average glucose value of

129.4 mg. per 100 cc. This value gradually decreased until at 2:00 P.M. the value was 86.6 mg. The calves were fed again at 4:30 P.M. and blood samples were drawn again at 5:00 P.M. At this time the average glucose value was 114.2 mg. The reason that this value did not equal the level of 129.4 mg., reached one hour after feeding in the morning, is undoubtedly due to the fact that only 30 minutes elapsed from the time of feeding until the blood sample was drawn in this case. By 11:30 P.M. the glucose value had decreased to 96 mg.

From this experiment it is readily seen that there is a marked diurnal variation in the blood sugar values of calves which is associated with feeding. Since the calves were being fed mainly milk, the high blood sugar values may easily be accounted for by the high lactose content of the diet.

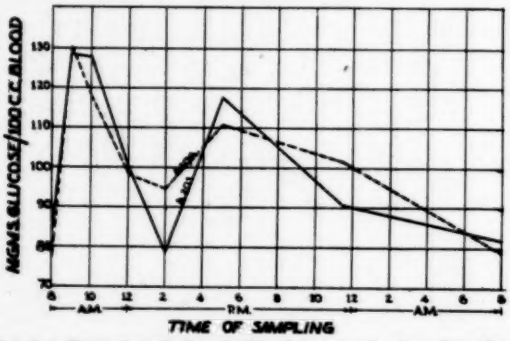

FIG. 2. Showing diurnal variation in blood sugar of calves less than one month of age.

Each calf received 13 pounds of milk a day which would contain about 0.6 pound of lactose. Thus at each feeding a calf received about 0.3 pound or 135 gm. of lactose. If in the case of a calf, milk passes directly into the abomasum and shortly into the intestine, there would be little delay in the digestion and absorption of lactose after milk is consumed.

When a carbohydrate tolerance test is given in human medicine the patient is given 1.75 gms. of glucose per kilogram of body weight. Since the average weight of the calves in this experiment was 87 pounds, the amount of sugar each received per feeding amounted to about 3.4 gms. per kilogram of body weight or about twice the amount given to a human in a sugar tolerance test. This would easily account for the rise in blood sugar after feeding. It is evident from this experiment that blood sugar values in calves must be interpreted on the basis of time of sampling in relation to time of feeding.

Sugar tolerance tests.—Although the feeding of large quantities of milk accounts in large measure for the high blood sugar values observed in the

above experiment, it does not account for the fact that even before feeding, calves have higher blood sugar values than mature cows.

In human nutrition it is recognized that the digestive mechanism is not highly developed in an infant. An infant may thrive on its mother's milk but may fail to develop properly on a modified cow's milk. This may be due to the fact that the infant has the digestive enzymes capable of handling human milk while it may be lacking in enzymes capable of digesting the slightly modified molecules in cow's milk. It is quite possible that the same situation holds with regard to the endocrine system of a young animal. If the pancreas of a young animal produces a limited supply of insulin, this might easily account for the high blood sugar in young calves. It is possible that a calf may have diabetic tendencies.

In human medicine, when a person is suspected of having diabetes, it is customary to make a carbohydrate tolerance test in which 1.75 gms. of glucose per kilogram of body weight is fed to the individual and the influence of such treatment on the blood sugar level is determined by frequent blood analyses. In order to determine whether or not calves have less ability to utilize sugar than cows, the following carbohydrate tolerance tests were made. The results are shown graphically in Figure 3.

Eighteen animals ranging in age from less than one month to mature cows were divided into eight groups on the basis of age as indicated in Figure 3. Each animal was given a carbohydrate tolerance test by feeding 1.75 gms. of glucose per kilogram of body weight administered as a drench in a 50 per cent solution.

Immediately before feeding the sugar meal blood samples were taken and analyzed for glucose. After the sugar meal, blood samples were drawn at the end of one, two, three and four hours and likewise analyzed for glucose. The values are plotted in Figure 3 for each animal.

It is evident from Figure 3 that animals less than one month of age show a much less rapid utilization of glucose than the animals of the older groups. As age increases there is better and better utilization of glucose until in the bred heifer and lactating cow groups there is only a slight elevation of blood sugar values after the sugar meal. Animals A–202 and A–187 do not appear to fit into the general picture. In the case of A–187 a possible explanation may be that this calf had been allowed to nurse different cows until it was four months of age and thereby may have developed the ability of taking care of more sugar than the other calves. It should also be noted that both A–202 and A–187 started the carbohydrate tolerance test at much lower blood sugar levels than their mates. It is possible that this low initial blood sugar level indicates a glycogen depletion at the outset of the test and that the sugar fed was used immediately for the production of glycogen. However the odd responses of these two animals may be more

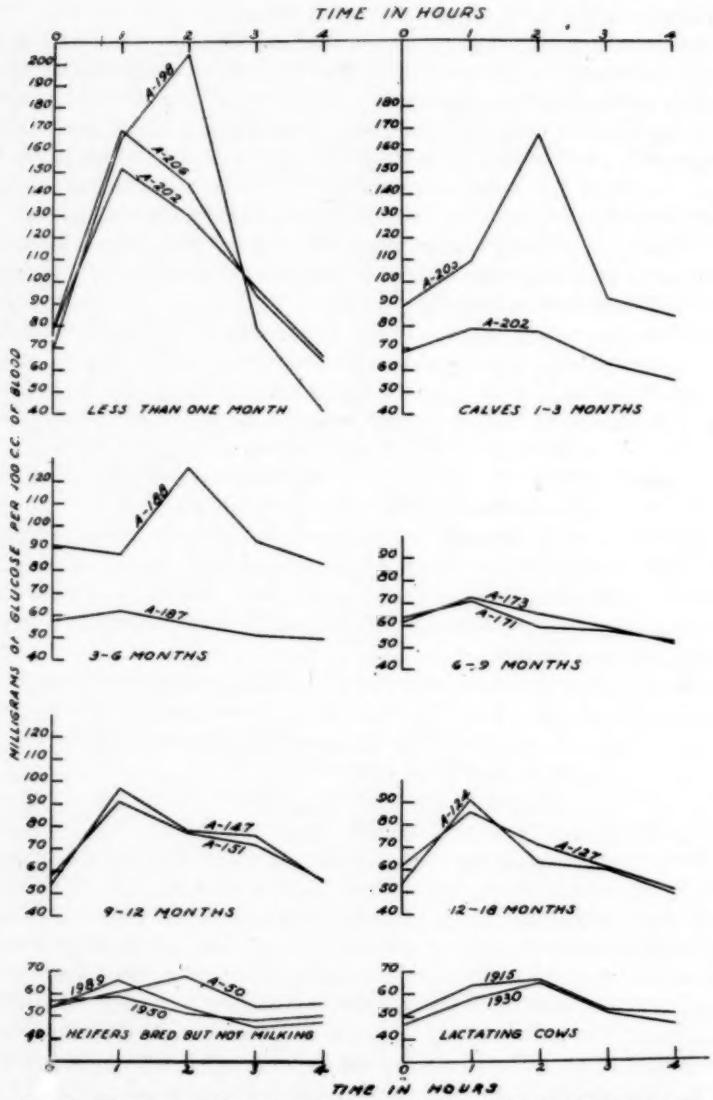

FIG. 3. Showing results of sugar tolerance tests on animals of various ages.

simply explained by assuming that they have developed an efficient glucose utilization mechanism at an early age.

From this experiment it is apparent that calves, especially very young calves, are less able to metabolize glucose than older animals. It would appear that the most logical explanation is that in very young calves the pancreas is not producing as much insulin as in older animals. It would be an interesting study to compare the insulin content of the pancreases of young calves and older cows. If it were found that the pancreases of older animals were richer in insulin than those of young animals this information might find a valuable application in the commercial manufacture of insulin.

SUMMARY

The blood of heifers has been analyzed at weekly intervals, from the time of breeding through one year of lactation, for non-protein nitrogen, glucose, calcium and phosphorus. There is little variation in these blood constituents as influenced by gestation and lactation. Average values for non-protein nitrogen were 32.82; for glucose 52.12; for calcium 10.91 and for phosphorus 5.44 mg. per 100 cc.

Blood glucose values are definitely related to age. Blood from calves less than one week of age contained 125 mg. of glucose per 100 cc. This value gradually decreased to 54.4 mg. per 100 cc. of blood at the end of a year.

There is a diurnal variation in the glucose content of calves' blood. Directly after feeding milk, blood sugar values increase sharply.

Carbohydrate tolerance tests on animals of various age groups indicate very little response to sugar feeding in the older groups but a decided response in young calves.

REFERENCES

(1) Anderson, A. K., Gayley, H. E., and Pratt, A. D. Studies on the chemical composition of bovine blood. J. Dairy Sc. 13: 336–348. 1930.

(2) Anderson, A. K., Honeywell, H. E., Santy, A. C., and Pedersen, S. The composition of normal rat blood. J. Biol. Chem. 86: 157–160. 1930.

(3) Awdejewa, M. S., Prowatorowa, E. L., Sawitsch, N. G., and Thal, E. L. Schwankungen des Blutzuckergahaltes beim Rind. Biochem. Zschr. 187: 369–376. 1927.

(4) Clark, E. P., and Collip, J. B. A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem. 63: 461–464. 1925.

(5) Fish, P. A. The physiology of milk fever. II. Hypoglycemia and normal blood sugar standards. J. Am. Vet. M. Ass., N.S. 26: 10–38. 1928.

(6) Folin, O. Two revised methods for blood sugar determinations. J. Biol. Chem. 82: 83–99. 1929.

(7) Folin, O., and Wu, H. A system of blood analysis. J. Biol. Chem. 38: 81–110. 1919.

(8) Haag, J. R. Calcium and inorganic phosphorus in the blood of dairy cattle. Pro-

ceedings of the 21st Annual Meeting, Western Division, Am. Dairy Sci. Assoc., 51–53. October, 1935.

(9) HAYDEN, C. E., AND FISH, P. A. The normal blood of some domesticated animals. Cornell Vet. 18: 197–203. 1928.

(10) HAYDEN, C. E., AND SHOLL, L. B. A study of the extractives of the blood of the cow. Report of the New York State Veterinary College at Cornell Univ., 102–110. 1923–24.

(11) HEWITT, E. A. The blood sugar level of the bovine. J. Am. Vet. M. Ass., N.S. 30: 362–367. 1930.

(12) HODGSON, R. E., RIDDELL, W. H., AND HUGHES, J. S. Factors influencing the blood sugar level of dairy cattle. J. Agr. Res. 44: 357–365. 1932.

(13) KILLIAN, J. A. Studies in diastatic activity of the blood and blood sugar curves indicating a decreased carbohydrate tolerance in hyperthyroidism. Proc. Soc. Exp. Biol. 17: 91–93. 1920.

(14) MEIGS, E. B., BLATHERWICK, N. R., AND CARY, C. A. Contributions to the physiology of phosphorus and calcium metabolism as related to milk secretion. J. Biol. Chem. 37: 1–75. 1919.

(15) MOUSSU, G., AND MOUSSU, R. Glycémie normale chez les bovidés. Glycémie et maladie vitulaire chez les vaches laitiéres. C. Rend. Acad. Sc. 183: 431–433. 1926.

(16) PETERS, J. P., AND VAN SLYKE, D. D. Quantitative clinical chemistry. Volume I, Interpretations, p. 285. The Williams and Wilkins Co., Baltimore. 1931.

(17) ROBINSON, C. S., AND HUFFMAN, C. F. Studies on the chemical composition of beef blood. I. The concentration of certain constituents in normal beef plasma. J. Biol. Chem. 67: 245–255. 1926.

(18) SCHEUNERT, A., AND V. PELCHRZIM, H. V. Über den Gehalt des Blutes verschiedener Tierarten an Zucker, Rest-N, Harnstoff-N, Kreatininkörpern und Harnsäure nach den Folinschen Methoden. Biochem. Zschr. 139: 17–29. 1923.

(19) SCHLOTTHAUER, C. F. Theories in the etiology of milk fever. Experimental investigations. Cornell Vet. 7: 217–224. 1928.

(20) SCHWARZ, K. Untersuchungen über den normalen Blutzuckergehalt des Pferdes und des Rindes. Biochem. Zschr. 194: 328–334. 1928.

(21) SCHWARZ, K., AND MEZLER-ANDELBERG, E. Untersuchungen über die Beziehung des Blutzuckergehaltes zur Milchproduction bei Kühen. Biochem. Zschr. 194: 362–375. 1928.

(22) TRABIN, B. A study of the glucose, non-protein nitrogen and chlorides in the blood of normal lambs and of lambs afflicted with the stiff-lamb disease. A thesis of the Graduate School of The Pennsylvania State College. 1937.

(23) WIDMARK, E. M. P., AND CARLENS, O. Über die Blutzuckerkonzentration bei Kühen und den Einflusz der Lactationsintensität auf dieselbe. Biochem. Zschr. 154: 454–459. 1925.

(24) YOUNGBURG, G. E., AND YOUNGBURG, M. V. Phosphorus metabolism. J. Lab. Clin. M. 14: 158–166. 1930.

CERTAIN FACTORS AFFECTING THE PHOSPHATASE TEST WHEN APPLIED TO ICE CREAM[1]

W. J. CAULFIELD AND W. H. MARTIN
Kansas Agricultural Experiment Station, Manhattan, Kansas

INTRODUCTION

The value of the phosphatase test as a control measure in the pasteurization of market milk has been well established in this country and abroad. Both experimental results and field studies indicate that even minor deficiencies in the pasteurization of milk can be detected with this test (1, 2, 3, 4, 5). The question arises as to the adaptability of the phosphatase test in detecting similar deficiencies in the pasteurization of ice cream mix. Except for the data of Hahn and Tracy (6) no experimental results have been published pertaining to the application of the test to ice cream.

Lack of information on the subject and the need for a quick accurate test for detecting improperly pasteurized samples of ice cream mix prompted the initiation of a research project on this subject. Factors studied were: (1) ingredients of the mix, (2) vanilla flavoring used, (3) variations in the time and temperature of pasteurization, (4) contamination of pasteurized mix with raw products, (5) homogenizing and freezing processes, and (6) storage of the frozen ice cream.

PROCEDURE

Two phosphatase tests, the Gilcreas and Davis test (3) and the New York field or vest pocket test (4) were used throughout this investigation. In addition the Scharer laboratory test (4) was used in a part of the study. The latter test, however, was discontinued because of the difficulty in filtering the ice cream mix samples and because the filtrates obtained were cloudy in many cases. A recent report on the Scharer test indicates that certain modifications in the technic and reagents may have overcome these difficulties (7).

The recommended procedures for each of the phosphatase tests were followed unless otherwise stated (3, 4). Near the completion of this study refinements in reagents and technic recently recommended were introduced in connection with the New York field test (7). These changes consisted in using a buffer substrate solution containing magnesium ions and the addition of neutralized butyl alcohol to the sample for the purpose of concentrating any blue color into the alcohol layer. Results obtained with this newer technic will be especially mentioned.

The ice cream mix used was calculated to contain 12 per cent of fat, 10

Received for publication October 29, 1938.

[1] Contribution No. 126, Department of Dairy Husbandry, Kansas State College.

per cent of serum solids, 15 per cent of sugar, and 0.35 of one per cent of gelatine. Ingredients used in preparing the mixes included raw cream, raw skim or raw whole milk, condensed or skim milk powder, sugar, and gelatine.

Commercial pasteurization was carried out in either a 100-gallon glass-lined pasteurizer or a 10-gallon steam-jacketed copper kettle equipped with a mechanical agitator. The mix temperature was checked by means of an indicating thermometer accurate to $\pm 0.2°$ F. The holding time was checked by a stop-watch and an interval timer. All samples taken during or at the conclusion of the pasteurization treatment were held in stoppered test tubes immersed in an ice water bath until tested.

The usual commercial procedure was followed in homogenizing, freezing, and hardening of the mixes when the influence of these factors on the results of the phosphatase test were studied.

When laboratory pasteurization was used it consisted of immersing test tubes containing samples of the mix being treated in a water bath maintained at the proper temperature. The mix temperature was checked by inserting an indicating thermometer (accurate to $\pm 0.2°$ F.) into a check tube of the mix. The holding time was controlled by the same timing devices mentioned in connection with commercial pasteurization.

EXPERIMENTAL RESULTS

Effect of various ingredients in the ice cream mix.—Sugar was found to exert a protective action against the inactivation of phosphatase when ice cream mixes were pasteurized commercially at 142, 143.5, and 145° F., respectively (Table 1). When mixes containing sugar were pasteurized at these temperatures for 30 minutes positive phosphatase results were obtained in all cases but when portions of these same mixes were pasteurized before the sugar was added, negative results were obtained.

TABLE 1

Influence of sugar on the inactivation of phosphatase in ice cream mixes as measured by two phosphatase tests when pasteurized at 142, 143.5, and 145° F., respectively, for 30 minutes. (Commercial pasteurization)

Pasteurization temperature (deg. F.)	Description of sample	Phosphatase test results (av. of 2 trials)	
		Gilcreas and Davis test*	New York field test
142	Complete mix	>0.150	+++
142	Mix without sugar	0.020	−−−
142	Sugar added after pasteurization	0.020	−−−
143½	Complete mix	>0.150	++
143½	Mix without sugar	0.020	−−
145	Complete mix	0.120	++
145	Mix without sugar	0.015	−−
145	Sugar added after pasteurization	0.015	−−

* Results expressed as mg. of phenol per 0.5 ml. of sample. Values in excess of 0.037 mg. of phenol are considered definitely positive.

The addition of sugar to the mixes after pasteurization, at 142 and 145° F. for 30 minutes, had no effect on the results of the phosphatase tests (Table 1). It is evident that the positive phosphatase reactions observed when mixes containing sugar were pasteurized at these temperatures were not due to the sugar itself but to the protective action of the sugar during heat treatment. A similar relationship between sugar and the destruction of bacterial cells was observed by Fay (8).

The data obtained in this study show that pasteurization of ice cream mixes containing sugar at 142–145° F. for 30 minutes was not adequate to inactivate the phosphatase present. Ice cream mixes thus pasteurized would all be adjudged as under-pasteurized on the basis of the phosphatase test. Furthermore, it would not be possible to detect differences in the pasteurization temperatures used within the limits 142–145° F. on the basis of the phosphatase test results (Table 1). These data as well as those obtained by Fay (8) indicate the necessity for using higher pasteurization temperatures with ice cream mixes than are ordinarily employed in the pasteurization of market milk.

Although sugar exerted a protective effect against the inactivation of phosphatase in mixes pasteurized at 142–145° F., no such effect was apparent when the pasteurization temperature was 150° F. (Table 2). Laboratory pasteurization at 150° F. for 30 minutes of various combinations of ice cream mix ingredients yielded negative phosphatase results in each of a series of three trials, irrespective of the presence of sugar (Table 2).

TABLE 2

Influence of ice cream mix ingredients on the inactivation of phosphatase as measured by three phosphatase tests when pasteurized at 150° F. for 30 minutes. (Laboratory pasteurization)

Ingredients used	Phosphatase test results (av. of 3 trials)		
	Gilcreas and Davis test*	Scharer laboratory test	New York field test
Milk and cream, raw	0.023	–†	–†
Milk, cream, and sugar	0.020	–	–
Milk, cream, and gelatine	0.020	–	–
Milk, cream, and condensed	0.020	–	–
Milk, cream, and powder	0.020	–	–
Complete mix	0.022	–	–

* Results expressed as mg. of phenol per 0.5 ml. sample. Values in excess of 0.037 are considered positive.

† Negative result.

Effect of vanilla flavors.—Ten different vanilla flavors (five pure bean, three reinforced, and two imitation) were used alone and in combination with ice cream mixes, and the samples tested with one or more phosphatase tests.

The pure bean vanillas, except for the concentrate, had no effect on results of the phosphatase test irrespective of the amounts used (Table 3).

When the pure bean vanilla concentrate was added directly to the buffer solution, a positive reaction to the New York field test was obtained. Also, a significant increase in the phenol value of the mix, as measured by the Gilcreas and Davis test, was obtained when double the recommended amount of flavor was added to the mix. When the pure bean vanilla concentrate was used according to the manufacturer's recommendations, however, both of the phosphatase tests yielded negative results.

Each of the reinforced or imitation vanilla flavors when added directly to the buffer solution gave a positive reaction by the New York field test. This observation is in harmony with the findings of Hahn and Tracy (6), who found that vanillin and coumarin resulted in positive reactions with the Scharer laboratory test. When used as recommended with ice cream mixes, only one of three samples (a reinforced vanilla) gave a positive reaction. This sample was positive only to the Gilcreas and Davis test.

When double the standard amount of reinforced or imitation vanillas were used, four of the five caused positive results with both phosphatase tests.

TABLE 3

Influence of certain vanilla flavors on the results of the phosphatase test.
(Average of two trials)

Sample number	Type of vanilla flavor used	Quantity of flavor used				
		1 drop to 5 cc. of buffer solution	Recommended amount for ice cream		Double the recommended amount for ice cream	
		Field test*	G & D test†	Field test	G & D test	Field test
1	Pure, concentrate	+	0.015	−	0.030	−
2	Pure	−	0.015	−	0.020	−
3	Pure	−	0.015	−	0.020	−
4	Pure	−	0.015	−	0.020	−
5	Pure	−	0.015	−	0.020	−
6	Reinforced	+	0.015	−	0.020	−
7	Reinforced	+	0.035	−	0.120	+
8	Reinforced	+	0.015	−	0.040	+
9	Imitation, concentrate	+	0.015	−	0.070	+
10	Imitation	+	0.015	−	0.060	+
11	Check (no flavor used)	−	0.015	−	0.020	−

* New York field test.
† Gilcreas and Davis test. Results expressed as mg. of phenol per 0.5 ml. sample. Values in excess of 0.037 are considered positive.

The data indicate that certain vanilla flavors, if used in excess, may cause a significant increase in the phenol value of an ice cream sample. It is important, therefore, to take into consideration the possibility of such interference when interpreting the results of phosphatase determinations on samples of vanilla ice cream.

Effect of variation in pasteurization temperature and time of holding.—

The results of six commercial and four laboratory pasteurization trials at 150° F. indicate that with but two exceptions the samples were negative to the Gilcreas and Davis phosphatase test at the end of a twenty-minute holding period (Table 4). In no case was a positive test obtained after a holding period of 25 minutes or more at 150° F.

The New York field test yielded negative results in all cases after a holding period of twenty minutes or more had elapsed, and with but two exceptions the results were negative after a 15-minute period (Table 4).

Mixes pasteurized at 160° F. were negative to the phosphatase tests after a five-minute holding period.

Variations noted in the time required for a mix to become negative to

TABLE 4

Effect of variations in pasteurization temperature and holding time on the inactivation of phosphatase in ice cream mixes

Holding time in minutes	Phosphatase test results											
	Gilcreas and Davis test						New York field test					
	Commercial pasteurization at 150° F.											
	Trial number						Trial number					
	1	2	3	4	5	6	1	2	3	4	5	6
0	++	++	++	++	++	++	++	++	++	++	++	++
5	++	++	++	+	++	++	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	−	−	+
15	−	+	+	−	−	+	−	−	−	−	−	−
20	−	−	−	−	−	+	−	−	−	−	−	−
25	−	−	−	−	−	−	−	−	−	−	−	−
30	−	−	−	−	−	−	−	−	−	−	−	−
	Laboratory pasteurization at 150° F.											
	1		2		3		4	1	2		3	4
0	++		++		++		++	++	++		++	++
10	+		+		+		+	+	+		+	+
15	+		−		+		−	+	−		+	−
20	−		−		+		−	−	−		−	−
25	−		−		−		−	−	−		−	−
30	−		−		−		−	−	−		−	−
	Commercial pasteurization at 160° F.											
	1	2	3	4	5	6	1	2	3	4	5	6
0	−	+	+	+	+	+	−	+	+	+	+	−
5	−	−	−	−	−	−	−	−	−	−	−	−
10	−	−	−	−	−	−	−	−	−	−	−	−
30	−	−	−	−	−	−	−	−	−	−	−	−
	Laboratory pasteurization at 160° F.											
	1	2	3	4	5	6	1	2	3	4	5	6
0	++	++	++	++	++	++	++	++	++	++	++	++
5	−	−	−	−	−	−	−	−	−	−	−	−
10	−	−	−	−	−	−	−	−	−	−	−	−
30	−	−	−	−	−	−	−	−	−	−	−	−

the phosphatase test when held at a specific temperature may be attributed largely to variations in the time required to heat the batch from 140° F. to the final pasteurization temperature. It is within this temperature zone that some phosphatase destruction occurs. Hence, the longer the time required to heat the mix through this temperature zone the lower will be the initial phosphatase content of the sample at the start of the holding period. This evidently explains why in several instances mixes pasteurized under laboratory conditions, where the heating takes place rapidly, remained positive to the phosphatase tests longer than did the mixes pasteurized with commercial equipment.

When ice cream mixes are pasteurized at 150° F. it is evident that the holding period might be shortened as much as 15 minutes before it would be detected with the Gilcreas and Davis phosphatase test. Similarly a reduction in the holding period of as much as 20 minutes could occur before it would be detected with the New York field test. In evaluating phosphatase test results on ice cream mixes it must be kept in mind, therefore, that a negative test does not mean that the mix has necessarily been held at 150° F. for a full thirty-minute period.

Similarly mixes pasteurized at 160° F. and held for five minutes would be adjudged efficiently pasteurized on the basis of the phosphatase test.

Detection of recontamination with a mixture of raw milk and cream.— In a series of six trials, the addition of 0.2 per cent or more of a mixture of raw milk and cream to a sample of pasteurized mix was detected in all cases with the Gilcreas and Davis test (Table 5).

With the New York field test, without the recently recommended refine-

TABLE 5

Sensitivity of phosphatase tests to the addition of raw milk and cream to pasteurized ice cream mixes

Sample number	Raw product added (per cent)	Phosphatase test results											
		Gilcreas and Davis						Field test (a)			Field test (b)		
		Trial No.						Trial No.			Trial No.		
		1	2	3	4	5	6	1	2	3	1	2	3
1	0.00	−	−	−	−	−	−	−	−	−	−	−	−
2	0.10	No samples			+	−	−	−	−	−	No samples		
3	0.20	+	+	+	+	+	+	−	−	−	−	−	−
4	0.30	+	+	+	+	+	+	−	−	−	+*	+*	+*
5	0.40	+	+	+	+	+	+	−	−	−	+*	+*	+*
6	0.50	+	+	+	+	+	+	+	+	−	+	+	+
7	0.75	+	+	+	+	+	+	+	+	+	+	+	+
8	1.00	+	+	+	+	+	+	+	+	+	+	+	+

* Samples gave a positive reaction only when butyl alcohol was used.

Field Test (a) = Original di sodium phenyl phosphate buffer substrate no butyl alcohol

Field Test (b) = New buffer substrate solution containing magnesium ions plus the use of butyl alcohol.

ments the presence of less than 0.75 per cent of added raw milk and cream was not detected in all cases. The use of the newer buffer substrate solution containing magnesium ions together with butyl alcohol in evaluating the final results increased the sensitivity of the New York field test to a point where 0.3 of one per cent of added raw milk and cream were detected without exception.

Effect of homogenization and freezing.—The phenol value of ice cream mixes taken before and after homogenization showed no significant changes, irrespective of whether the sample was negative or positive before treatment (Table 6). Similar results were obtained with samples of ice cream mix taken before and after freezing.

TABLE 6

Effect of homogenization on the phenol value of ice cream mixes as measured with the Gilcreas and Davis phosphatase test

Pasteurization treatment of mix	Number of trials	Phosphatase test results	
		Before homogenization (Ave. mg. phenol per 0.5 ml. sample)	After homogenization (2500 pounds' pressure) (Ave. mg. phenol per 0.5 ml. sample)
145° F. 30 min.	5	> .150	> .150
150° F. 30 min.	6	0.018	0.018
160° F. 30 min.	6	0.012	0.014*

* Sample taken at the completion of the freezing process, all other samples taken immediately before or after homogenization.

Effect of time and temperature of storage.—No marked change was observed in the phenol values of ice cream samples stored for two weeks in either an ice cream hardening room at approximately (−10° F.) or in a mechanically refrigerated cabinet at approximately (+10° F.) (Table 7). Longer storage periods than two weeks, however, have yielded somewhat variable results. Positive tests were observed with three additional sets of ice cream samples after a three weeks' storage period in both the cabinet and hardening room. All of these samples, however, were negative at the end of two weeks storage. In another trial, one of three sets of duplicate samples remained negative throughout a storage period of one month. Of the two other sets of samples one was positive in both the cabinet and hardening room, while only the sample maintained in the cabinet was positive in the remaining set after a month's storage period. No explanation for the factors responsible for these changes in the phenol values during storage can be given.

TABLE 7

Influence of time and temperature of storage on the phenol value of ice cream samples as measured with the Gilcreas and Davis phosphatase test

Pasteurization treatment of mix	Number of trials	Age of samples in weeks					
		Fresh		1		2	
		Place and temperature of storage					
		Cabinet (+10° F.)	Hardening room (−10° F.)	Cabinet (+10° F.)	Hardening room (−10° F.)	Cabinet (+10° F.)	Hardening room (−10° F.)
		Ave. phosphatase test results*					
145° F. 30 min.	5	>0.150	>0.150	>0.150	>0.150	>0.150	>0.150
150° F. 30 min.	8	0.023	0.024	0.027	0.027	0.027	0.027
160° F. 30 min.	5	0.017	0.017	0.023	0.021	0.023	0.024

* Values expressed as mg. of phenol per 0.5 ml. sample. Values in excess of 0.037 mg. of phenol are considered positive.

The results indicate that up to two weeks there is apparently little or no change in the phenol values of ice cream and that samples may be dependably tested any time during that period.

DISCUSSION

Results obtained in this study support the conclusions of Hahn and Tracy that the phosphatase test, with certain limitations, may be used as an index to satisfactory pasteurization of ice cream or ice cream mixes. Since one of these limitations is the possible effect of imitation or reinforced vanilla flavors on the results of the tests, it would be desirable, whenever possible, to apply the test to the unflavored mix. If this is not possible, the precaution should be taken to run a suitable control test on all positive samples to avoid misinterpretation of the results.

A negative phosphatase test on a sample of mix does not necessarily mean that the sample has been pasteurized at 150° F. for 30 minutes, which is the legal requirement for the pasteurization of ice cream mixes now in effect on many markets. Negative phosphatase results were obtained in this study after a holding period of 15 to 25 minutes at this temperature. Similarly a holding period of 5 minutes proved sufficient to inactivate phosphatase when a pasteurization temperature of 160° F. was used. Hence, it would be difficult to enforce a legal standard requiring a pasteurization temperature of 150 or 160° F. for 30 minutes with the phosphatase tests used in this study. On the other hand, results obtained in this study show that the phosphatase test is capable of detecting mixes which have not been heated in excess of 145° F. for 30 minutes.

The fact that the holding period with pasteurization temperatures of 150 or 160° F. could be materially shortened before it would be detected with either of the phosphatase tests used in this study raises the question as to just what constitutes efficient pasteurization of ice cream mixes. Can a mix which is negative to the phosphatase test be considered efficiently pasteurized even though it may have been held for only 15 minutes at 150° F. or 5 minutes at 160° F.? Obviously the true answer to the question must be determined on the basis of the bacteriological flora surviving such treatments. If such studies demonstrate that the mix is pasteurized satisfactorily from a bacteriological viewpoint before it becomes negative to the phosphatase test, then the value of the test as a true index to the satisfactory pasteurization of ice cream mixes will be greatly increased. Until the results of such a study are known, however, it would be unsafe to assume that negative phosphatase tests are necessarily indicative of proper pasteurization. On the other hand, positive tests would always be indicative of inefficient pasteurization.

Both the New York field and Gilcreas and Davis tests were found to be extremely sensitive to the addition of raw products to a sample of properly pasteurized mix. The tests should prove extremely useful in detecting the indiscriminate dilution of properly pasteurized mix with raw milk or cream.

Although not perfect, the phosphatase tests used in this study offer greater possibilities for insuring adequate pasteurization of ice cream than has been available up to the present time.

SUMMARY AND CONCLUSIONS

A study has been completed to determine whether or not the phosphatase test can be satisfactorily used to determine the pasteurization efficiency of ice cream. Factors studied were: (1) ingredients, (2) vanilla flavors, (3) variations in the time and temperature of treatment, (4) contamination of pasteurized mix with raw products, (5) homogenization and freezing, and (6) storage of the frozen ice cream.

The more important results of this study may be summarized as follows:

1. Sugar in the mix was found to protect phosphatase against inactivation by heat when pasteurization temperatures of 142, 143.5 and 145° F. for 30 minutes respectively were used. Portions of these same mixes, but pasteurized without sugar, gave negative phosphatase reactions at these same temperatures.

2. When the pasteurization temperature was increased to 150° F. for 30 minutes negative phosphatase results were observed in all cases irrespective of the presence of sugar in the mix.

3. Certain vanilla flavors if used in excess were found to cause a significant increase in the phenol value of an ice cream mix.

4. The data show that the usual 30 minute holding period may be

materially shortened before it would be detected by either the Gilcreas and Davis, or New York field phosphatase tests when ice cream mixes are pasteurized at 150° F. or higher.

5. It was possible with the Gilcreas and Davis test to detect the admixture of as little as 0.2 per cent added raw milk and cream to a sample of pasteurized mix.

Seventy-five hundredths of one per cent added raw milk and cream could be detected with the original New York field test and with a modified technic it was possible to detect the presence of 0.3 per cent added raw products.

6. No differences were observed in the results of the phosphatase tests as a result of homogenization or freezing.

7. Samples of ice cream stored for two weeks in an ice cream cabinet (at approximately + 10° F.) and in an ice cream hardening room (at approximately – 10° F.) showed no significant change in their phenol values.

REFERENCES

(1) KAY, H. D., AND GRAHAM, W. R. The phosphatase test for pasteurized milk. J. Dairy Res. 6: 191–203. 1935.

(2) GILCREAS, F. W., AND DAVIS, W. S. Investigation of the amylase and phosphatase tests as an indication of pasteurization. Proc. Intl. Assoc. Milk Sanitarians, p. 15–31. 1936.

(3) GILCREAS, F. W., AND DAVIS, W. S. The practical value of the phosphatase test in determining the efficiency of pasteurization. Annual Proc. Intl. Assoc. Milk Dealers, Laboratory Section, p. 34–56. 1937.

(4) SCHARER, H. A rapid phosphomonoesterase test for control of dairy pasteurization. J. DAIRY SC. 21: 21–33. 1938.

(5) HAHN, A. H., AND TRACY, P. H. Determining the efficiency of milk pasteurization. Annual Proc. Int. Assoc. Milk Dealers, Lab. Sect., p. 57–71. 1937.

(6) HAHN, A. H., AND TRACY, P. H. Application of the phosphatase test to determine the efficiency of pasteurization of ice cream mix. J. DAIRY SC. 21: 158.

(7) SCHARER, H. Improvements in the rapid phosphatase test for detection of improper pasteurization of milk and its products. J. Milk Tec. 1: 35–38. July, 1938.

(8) FAY, A. C. The effect of hypertonic sugar solutions on the thermal resistance of bacteria. J. Dairy Res. 48: 453–468. 1934.

ASCORBIC ACID AND OXIDIZED FLAVOR IN MILK. I. DISTRIBUTION OF ASCORBIC ACID AND OCCURRENCE OF OXIDIZED FLAVOR IN COMMERCIAL GRADE A RAW, IN PASTEURIZED IRRADIATED, AND IN PASTEURIZED MILK THROUGHOUT THE YEAR[1]

G. M. TROUT AND ERLAND C. GJESSING

Recent developments in the chemical aspects of vitamin C, in its isolation, and in the exact chemical methods for its determination have made possible rapid and accurate methods for determining the amount of vitamin C as ascorbic acid in a great variety of foods. The determination of ascorbic acid in milk by chemical methods has recently been made possible.

Sharp (5), using the rapid 2–6 dichlorophenolindophenol titration method (6) for the determination of reduced ascorbic acid, showed that the ascorbic acid of milk holder pasteurized in glass was as stable as that in the raw product similarly handled to prevent metal contamination. He also demonstrated (4) the high correlation which existed between the presence of the oxidized flavor and the almost total lack of ascorbic acid in milk.

Whitnah *et al.* (8) observed an inverse relationship between the naturally occurring vitamin C in breed milk and the frequency of the development of the oxidized flavor. However, they found no relationship between the amount of vitamin C and the development of oxidized flavor in milk from individual cows.

Rasmussen *et al.* (2) mentioned the apparent relation between the "cardboard" flavor and a low ascorbic acid content of milk. They observed that samples of milk which developed cardboard flavor on standing invariably decreased in ascorbic acid coincident with the formation of the off-flavor.

Inasmuch as this oxidized flavor is usually a seasonal problem, disappearing in the late spring and recurring in the fall and winter, an understanding of the rôle of ascorbic acid in commercial milk throughout the year seemed desirable. Consequently, the major portion of the data reported herein was obtained by routine analysis from daily, bi-weekly, or tri-weekly samples of commercially processed milk throughout the year.

EXPERIMENTAL

Samples of grade A raw, pasteurized irradiated, and regular pasteurized milk taken daily, bi-weekly, or tri-weekly throughout the year were titrated for ascorbic acid daily for four days. The milk was titrated with 2–6 dichlorophenolindophenol using the rapid method of Sharp (6).

The grade A raw milk, which averaged approximately 4.5 per cent fat,

Received for publication October 29, 1938.

[1] Authorized as Mich. Agri. Exp. Sta. Jour. Article No. 224 (n.s.).

was produced at the College Dairy Barn. Here the milk was machine drawn into aluminum containers from which it was poured over a surface cooler; stored in 10-gallon cans; the noon, night, and morning's milk mixed and bottled daily in a rotary bottler.

The irradiated milk was essentially the same as the grade A raw milk with the exception that it was irradiated in a CP irradiator and then pasteurized at 142°–143° F. for 30 minutes.

The regular pasteurized mixed milk, testing 3.7 per cent fat, was obtained from approximately 30 producers delivering milk to the College Creamery. The milk was holder pasteurized in glass-lined or in Allegheny metal pasteurizers.

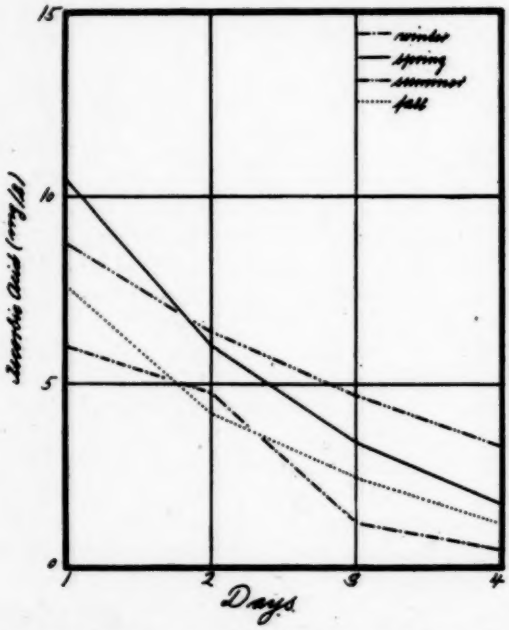

FIG. 1. Seasonal variation of ascorbic acid in grade A raw milk after different periods of storage.

Pint samples of the stored bottled milk were secured at random at time of loading and delivery and portions from each bottle were titrated for ascorbic acid each day for four successive days. The milk obtained for titration, therefore, was night's and morning's milk mixed, processed, and stored for distribution the following day. Consequently, the age of the milk at time of the first day's titration was from 24 to 34 hours.

TABLE 1

Percentage distribution of samples of grade A raw milk according to ascorbic acid content by seasons

Ascorbic acid	Grade A raw milk held four days at 40° F.; percentage distribution of															
	Winter samples				Spring samples				Summer samples				Fall samples			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
Mg. per liter	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0– 0.99		7.1	61.0	75.1		11.1	18.5	36.7			3.3	12.9	2.9	3.2	22.3	54.6
1.00– 1.99	3.3	21.4	16.7	18.8	2.3	11.1	22.2	33.3			13.3	19.4	5.9	19.4	33.3	27.3
2.00– 2.99		7.2	5.6			8.3	3.7	10.0	4.4	15.8	16.6	25.8	11.7	22.5	22.2	9.1
3.00– 3.99	3.3	14.3	5.6	6.3	4.7	2.8	11.1	6.7	8.9	5.3	13.3	6.4		16.1	5.6	9.1
4.00– 4.99	13.3	7.2	5.6		7.0	13.9	14.8	10.0	8.9	13.2	6.7	9.7	5.9	12.9	5.6	
5.00– 5.99	6.7	10.7	5.6		2.3	5.6	14.8		2.2	15.8	10.0	12.9	5.9	3.2		
6.00– 6.99	6.7	17.9			2.3	5.6	7.4		11.1	15.8	20.0	3.2	5.9	3.2		
7.00– 7.99	13.3				4.7	8.3	3.7	3.3	6.8	2.6	3.3	9.7	8.8	6.6	5.6	
8.00– 8.99	13.3	7.2			7.0	5.6	3.7		4.4	13.2	6.7		20.6		5.6	
9.00– 9.99	13.3	3.6			9.3	11.1			11.1	5.3	3.3		2.9	3.2		
10.00–10.99	6.7	3.6			9.3	8.3			6.8	2.6	3.3		2.9	3.2		
11.00–11.99	3.3				14.0	2.8			11.1	7.9			8.8	3.2		
12.00–12.99	3.3				2.3	5.6			2.2	2.6			5.9	3.2		
13.00–13.99	10.0				11.6				2.2				5.9			
14.00–14.99	3.3				11.6				13.3				2.9			
15.00–15.99					7.0				4.4							
16.00–16.99					2.3				2.2				2.9			
17.00–17.99					2.3											
No. of samples	30	28	18	15	43	36	27	30	45	38	30	31	34	31	18	11

RESULTS

The percentage distributions of the samples of grade A raw, pasteurized irradiated, and regular pasteurized milk, according to ascorbic acid content, by seasons, are given in Tables 1, 2, and 3. The average seasonal values, presented in Table 4, are shown graphically in Figures 1, 2, and 3. The percentage decreases in ascorbic acid and the incidence of oxidized flavor during the seasons are given in Table 4.

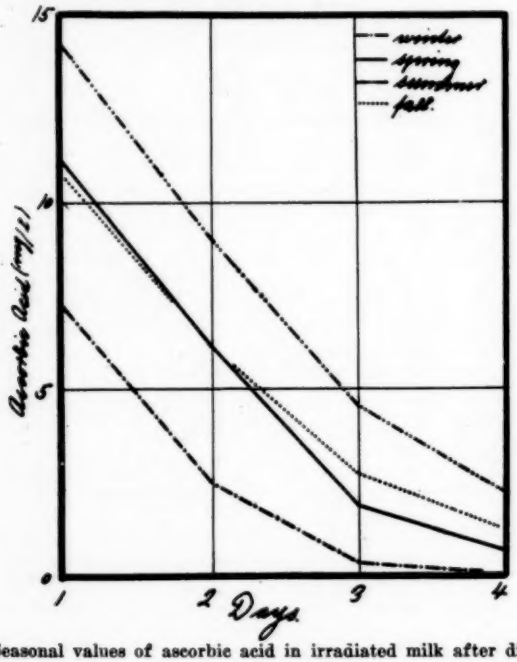

FIG. 2. Seasonal values of ascorbic acid in irradiated milk after different periods of storage.

Grade A raw milk.—The percentage distribution of samples of grade A raw milk by ascorbic acid content and by season is shown in Table 1. A comparison of the distribution values for the third and fourth day's titration for winter, spring, summer and fall respectively shows the relatively high percentage of the samples having little or no ascorbic acid during the fall and winter months and the slightly greater amounts of ascorbic acid present in milk held for similar periods during the spring and summer months. However, as shown in Table 4, on the first day of titration, 6.0, 10.5, 8.8, and 7.7 mg. per liter of ascorbic acid were present in the grade A milk during the winter, spring, summer and fall seasons respectively. The average ascorbic acid value for the first day throughout the year was 8.3

TABLE 2

Percentage distribution of samples of pasteurized irradiated milk according to ascorbic acid content by seasons

Ascorbic acid	Pasteurized irradiated milk held four days at 40° F.; percentage distribution of															
	Winter samples				Spring samples				Summer samples				Fall samples			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
Mg. per liter	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0– 0.99		18.0	46.7	100.0		4.8	11.4	71.5			2.9	14.3		10.7	25.0	52.6
1.00– 1.99	2.3	25.6	43.3				14.3	14.3		2.4		25.7			10.0	26.3
2.00– 2.99	4.5	10.3	10.0			4.8	31.4	7.1			20.0	37.1		3.6	15.0	10.5
3.00– 3.99		28.2				7.1	17.1	4.6		2.4	20.0	14.3		3.6	25.0	10.5
4.00– 4.99	4.5	17.9				9.5	11.4	2.3			14.3	5.7		10.7	15.0	
5.00– 5.99	18.2					14.3	5.7			2.4	17.1	2.8	2.9	10.7	5.0	
6.00– 6.99	9.1					23.8	5.7			9.6	11.4			28.6		
7.00– 7.99	25.0					19.0				14.4	5.7		2.9	14.3		
8.00– 8.99	18.2				2.0	9.5	2.9			16.8	2.9		11.7	3.6	5.0	
9.00– 9.99	11.4				2.0	2.4				16.8	2.9		14.7	3.6		
10.00–10.99	6.8				16.3	4.8				16.8	2.9		23.5	3.6		
11.00–11.99					18.4				8.7	4.8			11.7	7.2		
12.00–12.99					28.6				16.7	4.8			17.6			
13.00–13.99					18.4				21.7	2.4			2.8			
14.00–14.99					12.2				15.2				5.9			
15.00–15.99					2.0				19.4	2.4			5.9			
16.00–16.99									13.0							
17.00–17.99									4.3							
No. of samples	44	39	30	33	49	42	35	43	46	41	35	35	34	28	20	19

TABLE 3

Percentage distribution of samples of regular pasteurized milk according to ascorbic acid content by seasons

Ascorbic acid	Pasteurized milk held four days at 40° F.; percentage distribution of															
	Winter samples				Spring samples				Summer samples				Fall samples			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
Mg. per liter	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0– 0.99		2.6	14.2	63.6			9.4	15.2				3.0				29.4
1.00– 1.99		5.1	14.3	22.7		7.0	9.4	36.4			3.1	6.1			5.3	11.8
2.00– 2.99		10.3	25.0	4.5			6.3	12.1		2.6	6.2	33.3		3.3	15.8	11.8
3.00– 3.99	2.3	7.7	17.9	4.5		4.6	18.8	30.4			9.4	21.2			10.5	5.9
4.00– 4.99		10.3	7.2	4.5		2.3	9.4				18.7	24.2		10.0	10.5	11.8
5.00– 5.99	4.5	12.8	7.2			4.6	18.8			2.6	15.6	9.1		3.3	5.3	5.9
6.00– 6.99	9.1	15.4	10.7			9.3	15.6	3.0		2.6	12.5	3.0	2.6	10.0		
7.00– 7.99	9.1	12.8	3.6		1.9	23.2	9.4	3.0		7.7	18.7		5.3	3.3	21.1	11.8
8.00– 8.99	2.3	17.9				25.6				15.4	12.5			10.0		11.8
9.00– 9.99	18.2	5.1			3.9	11.6	3.1			28.2			15.8	16.7	5.3	
10.00–10.99	15.9				3.9	7.0				20.5	3.1		2.6	3.3	21.1	
11.00–11.99	13.6				15.7	2.3			6.8	12.8			5.3	6.6	5.3	
12.00–12.99	20.5				31.4				9.1	5.1			7.9	26.6		
13.00–13.99	4.5				15.7				20.5	2.6			18.4	6.7		
14.00–14.99					11.8	2.3			43.2				10.5			
15.00–15.99					11.8				13.6				18.4			
16.00–16.99					1.9				4.5				7.9			
17.00–17.99					1.9				2.3				5.3			
No. of samples	44	39	28	22	51	43	32	33	45	39	32	33	38	30	19	17

mg. per liter which was a lower average than that found in either the irradiated milk or in the regular pasteurized milk, under similar storage conditions. Graphical presentation of these data is shown in Figure 1. Here it will be observed that the amount of ascorbic acid in grade A raw milk was consistently higher in spring and summer than in the fall and winter. The percentage decrease of ascorbic acid upon storage was greater in the winter than in the spring, summer, or fall. The percentage decreases

FIG. 3. Seasonal values of ascorbic acid in pasteurized milk after different periods of storage.

of ascorbic acid, after three days' storage during these seasons were respectively 78.3, 57.6, 46.5 and 67.5 (Table 4).

From late fall and throughout the winter, the grade A raw milk was occasionally criticized for its oxidized flavor. Organoleptic examinations revealed during this period slight to distinct oxidized flavor developments when the milk was held from 48 to 72 hours, but no oxidized flavors were noted in this milk during spring and summer. The grade A raw milk was produced free from copper contamination, and under sanitary conditions which resulted in average bacterial counts below 10,000 per cc.

Pasteurized irradiated milk.—The percentage distribution of the samples of pasteurized irradiated milk by ascorbic acid content and by season is

shown in Table 2. During the winter months, 90 per cent of the samples showed less than 2.0 mg. per liter of ascorbic acid remaining on the third day of titration. On the fourth day the ascorbic acid had disappeared entirely from all the samples. As shown in Table 4, little difference was noted in the amount of ascorbic acid present in the spring and fall; however, at the third and fourth titration both were notably low. The ascorbic

TABLE 4

Average seasonal values of ascorbic acid in grade A raw, in pasteurized irradiated, and in pasteurized milk with the oxidized flavor noted

Season	Ascorbic acid mg. per liter at				Per cent decrease in ascorbic acid from 1st to 3rd day at which time samples were examined for flavor	Oxidized flavor development usually noted after 3 days*
	1st day	2nd day	3rd day	4th day		
Grade A milk						
Winter	6.0	4.8	1.3	0.6	78.3	++
Spring	10.5	6.0	3.4	1.8	57.6	–
Summer	8.8	6.4	4.7	3.3	46.5	–
Fall	7.7	4.2	2.5	1.2	67.5	+
Average	8.3	5.4	3.0	1.7		
Pasteurized irradiated milk						
Winter	7.2	2.5	0.4	0.0	94.4	+++
Spring	11.1	6.1	2.0	0.7	81.9	+
Summer	14.2	9.1	4.7	2.2	66.9	–
Fall	10.9	6.1	2.8	1.2	74.3	+
Average	10.9	6.0	2.5	1.0		
Pasteurized milk						
Winter	9.9	5.8	3.1	0.8	68.7	–
Spring	12.9	7.6	4.0	2.2	68.9	–
Summer	13.0	9.5	5.8	3.3	55.4	–
Fall	13.0	9.4	6.8	3.4	47.7	–
Average	12.2	8.1	4.9	2.4		

* Key: – no oxidized flavor
? doubtful oxidized flavor
+ slight oxidized flavor
++ distinct oxidized flavor
+++ very strong oxidized flavor

acid content of summer milk was low also on the third and fourth days of titration, but was higher than that observed at the other seasons.

The first day values for ascorbic acid in irradiated milk throughout the year were 7.2, 11.1, 14.2, and 10.9 mg. per liter for the winter, spring, summer and fall seasons respectively, these values being higher than those found in grade A milk under similar storage (Table 4). The values graphically presented in Figure 2 show that the average ascorbic acid content per day of storage in summer milk was approximately as much above that of spring and fall milk as that in winter milk was below the spring and fall values.

Again, the percentage decrease of ascorbic acid in stored winter milk was higher than that observed during the other seasons. The percentage seasonal decreases of ascorbic acid from the first to the third day of storage were found to be 94.4, 81.9, 66.9 and 74.3 for the winter, spring, summer and fall seasons, respectively.

From November 1st until April 1st this milk consistently developed upon storage an oxidized flavor which was readily noticeable 48 hours after bottling by those inexperienced in detecting flavor. The flavor was stronger than that encountered in the grade A milk during the same period. Inasmuch as the irradiated milk had a slightly higher initial ascorbic acid content which disappeared at a greater rate than that in the grade A milk (Table 4), it appeared that the ascorbic acid of this milk was affected by some catalyst not present in the grade A raw milk. Since this milk was irradiated, the natural conclusion would be that the ultraviolet light was exerting the major catalytic effect. However, later work showed that some factor other than irradiation was responsible for the high decrease in ascorbic acid. Copper was evidently responsible as this milk contacted some exposed copper on the spreader trough prior to irradiation. Application of Herrington's test (1) for copper in the metal suspected yielded positive results.

The apparent relation between the percentage decrease of and almost total lack of ascorbic acid and the development of the oxidized flavor seems worthy of note. If ascorbic acid is a reducing substance, then, it appears that appreciable oxidation of the fatty constituents may not be expected in the presence of significant amounts of it. When a distinct oxidized flavor was present, titration showed the presence of little or no ascorbic acid. On the contrary, many samples have been noted in which negligible quantities of ascorbic acid were present, yet there was no development of the oxidized flavor. Such observations were made more frequently during the spring and summer than during the fall and winter. The high stability of summer milk against oxidized flavor development, even with copper contamination within the plant, is well recognized commercially.

Pasteurized milk.—The percentage distribution of the samples of regular pasteurized milk by ascorbic acid content and by season is presented in Table 3. With few exceptions, the regular pasteurized milk throughout the year had a higher ascorbic acid value than did either the grade A raw or the irradiated pasteurized milk, on any of the days of titration. The ascorbic acid content of this milk, as with the other grades, disappeared almost entirely by the fourth day of titration during the winter months. However, the ascorbic acid content of the pasteurized milk during the winter months on the first day's titration was higher than that found in the grade A or in the irradiated milk (Table 4).

The seasonal variations of ascorbic acid in the pasteurized milk, with

the exception of during the winter, was not exhibited to any appreciable extent by the results of the first day's titration. These titrations for the winter, spring, summer and fall months gave average ascorbic acid values of 9.9, 12.9, 13.0 and 13.0 mg. per liter, respectively.

The ascorbic acid values obtained for each day's titration, presented graphically in Figure 3, show that the ascorbic acid content of the pasteurized milk was consistently lower in the winter and, after the first day's titration, was lower in the spring than in the summer and fall. Little difference was noted in the amount of ascorbic acid present during these last two seasons.

Of the three milks included in this study, grade A raw, irradiated pasteurized and regular pasteurized, the regular pasteurized was the only kind which did not develop oxidized flavors during the winter season. The milk was pasteurized in glass-lined and in Allegheny metal vats and was conveyed to the bottler through Allegheny metal pipes. The cabinet cooler and distributor were tinned copper, but no exposed copper was noted.

Inasmuch as night's and morning's milk were processed in the forenoon prior to delivery early the following morning, 24 to 34 hours necessarily had elapsed between time of production and time of titration. It must follow, therefore, that the lack of agreement between the lower ascorbic acid values found generally in milk in these studies, grade A, irradiated pasteurized and pasteurized, in comparison with those reported (3, 4, 7) in milk titrated within a few hours after milking, must be due in large part to the factors involved during the lapse of time between the respective titrations.

First days' titrations in these studies represent titration of the milk on the day of distribution, not after it had been held further for another day. Consequently, the ascorbic acid values found on the first titration should represent closely the ascorbic acid value of delivered milk, when the milk is produced and distributed under the conditions of the study.

SUMMARY

Samples of commercial grade A raw, irradiated pasteurized, and regular pasteurized milk, taken daily, bi-weekly, or tri-weekly, at time of delivery throughout the year, were titrated daily for four days for ascorbic acid. The 2–6 dichlorophenolindophenol direct titration method of Sharp (6) was used in determining the ascorbic acid values. On the third day of titration the milk was examined for development of the oxidized flavor.

The ascorbic acid content of winter milk was lower than that of spring, summer, or fall milk on the first, second, third or fourth day of storage. Summer milk under conditions of the study usually had more ascorbic acid than spring and fall milk under similar conditions of study, especially on the third and fourth days. Ascorbic acid values for spring and fall milk generally were between the summer and winter values, but varied as to the period of storage.

The average first day values of ascorbic acid in the grade A milk studied were 6.0, 10.5, 8.8, and 7.7 milligrams per liter during winter, spring, summer and fall, respectively. Those for pasteurized irradiated milk were 7.2, 11.1, 14.2, and 10.9 milligrams per liter while those for regular pasteurized milk were 9.9, 12.9, 13.0 and 13.0 milligrams per liter during the same respective seasons.

The rate of disappearance of ascorbic acid, calculated on the third day of titration, when the milk was examined for oxidized flavor, was greater in the winter than in the summer, being relatively greater in the irradiated and in the grade A milk than in the pasteurized milk. Conversely, the stability of ascorbic acid against oxidation was greater in summer than in winter milk.

The stored irradiated milk had a strong oxidized flavor from late fall to early spring. The grade A raw milk had a less pronounced oxidized flavor during this period, but was yet troublesome, whereas no oxidized flavor was noted in the pasteurized milk throughout the year.

The direct titration method of determining the ascorbic acid in milk was found to be very helpful in predicting the presence of a catalyst in commercial bottled milk.

REFERENCES

(1) Herrington, B. L., and Brereton, John G. A simple method for detection of copper in alloys. J. Dairy Sc. 20: 197. 1937.

(2) Rasmussen, R., Guerrant, N. B., Shaw, O. A., Welch, R. C., and Bechdel, S. I. The effects of breed characteristics on the vitamin C (ascorbic acid) content of cow's milk. J. of Nutrit. 11: 425. 1936.

(3) Riddell, W. H., Whitnah, C. H., Hughes, J. S., and Lienhardt, H. F. Influence of the ration on the vitamin C content of milk. J. of Nutrit. 11: 47. 1936.

(4) Sharp, P. F., Trout, G. M., and Guthrie, E. S. Vitamin C, copper and oxidized flavor of milk. Tenth Ann. Report N. Y. State Assoc. of Dairy and Milk Inspectors, pp. 153–164. 1936.

(5) Sharp, P. F. Vitamin C in pasteurized milk. Science 84: 461–462. 1936.

(6) Sharp, P. F. Rapid method for the quantitative determination of reduced ascorbic acid in milk. J. Dairy Sc. 21: 85–87. 1938.

(7) Whitnah, C. H., Riddell, W. H., and Caulfield, W. J. The influence of storage, pasteurization, and contamination with metals on the stability of vitamin C in milk. J. Dairy Sc. 19: 373. 1936.

(8) Whitnah, C. H., Martin, W. H., and Beck, G. H. Oxidized milk flavor as related to carotene, lecithin, and vitamin C. J. Dairy Sc. 20: 431. 1937.

MAGNESIUM AND VITAMIN D RELATIONSHIPS IN CALVES FED MINERALIZED MILK

C. E. KNOOP, W. E. KRAUSS, AND C. C. HAYDEN

Department of Dairy Industry, Ohio Agricultural Experiment Station, Wooster, Ohio

It has been shown from previous work (1) that dairy calves will develop an anemia when fed whole milk alone which can be prevented by feeding iron and copper salts. It was observed that after these salts were added there still existed deficiencies which appeared after a majority of the calves were 120 to 150 days of age, for they would stand on their toes, with their heads in a lowered position and their backs slightly arched. Many of the bones from these calves were weak, soft, hemorrhaged in areas, and low in magnesium. These observations stimulated further work with calves receiving a limited amount of whole milk supplemented in various ways.

REVIEW OF THE LITERATURE

Experiments which have been done with dogs and rats show conclusively that magnesium is an essential mineral for health and growth (2, 3, 4, 5, 6, and 7).

During the progress of our present experiment, Duncan, Huffman, and Robinson (8) published data showing that calves may succumb to magnesium tetany at almost any time after 30 days of age when fed whole milk alone or supplemented with various combinations of iron, copper, manganese, and cod-liver oil.

Later, Huffman and Duncan (9) fed a group of calves a rachitogenic basal ration and studied the value of adding cod-liver oil, alfalfa hay, magnesium carbonate, and magnesium oxide. Their results showed that when a small amount of vitamin D and a magnesium supplement were incorporated into the rachitic ration rickets did not appear. However, rickets would appear when a magnesium supplement was not fed. They also found, in general, that when calves were fed a rachitogenic ration supplemented with a small amount of cod-liver oil and magnesium the calcium and phosphorus blood picture was maintained at normal up to 10½ months of age.

EXPERIMENTAL

Three groups of three Holstein calves each were used in the present studies. The calves in Group 1 (calves F 549, M 455, and M 480) received daily a basal ration containing a limited amount of whole milk fortified with 400 mg. of iron as iron chloride, and 40 mg. of copper as copper sulfate. Group 2 (calves F 543, M 472, and M 483) received in addition to the basal ration magnesium carbonate at the rate of 1 per cent of the dry matter

Received for publication September 29, 1938.

content of the milk fed. The calves in Group 3 (calves F 562, M 478, and M 484) received in addition to the basal ration magnesium carbonate at 1 per cent of the dry matter content of the milk fed and vitamin D from ultraviolet light.

The milk used in this experiment was produced by cows fed alfalfa hay, corn silage or wet beet pulp, and a regular grain mixture. The rate of milk feeding was determined by that consumed by the calves in a previous test (1). This amount varied monthly from 10 to 16.5 pounds per day as the experiment progressed.

Vitamin D was supplied by irradiating the calves for 60 minutes daily with a General Electric S_1 Sunlamp, which was suspended 30 inches above the backs of the calves. The ultraviolet output of the lamps was 10 to 35 microwatts per square centimeter, an amount which had been found sufficient to cure severe rickets in calves fed a rachitogenic ration.

All calves were muzzled throughout the experiment.

Liveweights and measurements of the height at withers were taken at birth and every 2 weeks thereafter. Calcium (10), phosphorus (11), and magnesium (12) contents of the blood serum were determined when the calves were 12 and 13 days of age and at 30-day intervals thereafter. All calves were killed when 255 days of age and the metacarpal, metatarsal, and fifth rib bones removed. All adhering flesh was removed from these bones, and many of them were photographed. The following gross measurements were made of the left metacarpal and metatarsal bones: green weight, length, smallest diameter, width of epiphysis, volume, density, and breaking strength. After breaking, the left leg bones of each calf were ground together and aliquoted. Both fifth ribs from each calf were ground together. All samples were extracted with hot alcohol for 72 hours, and then with ether for 18 hours in Soxhlet extractors. Calcium (13) and magnesium (14) were determined in the bone ash. Phosphorus (15) and bone ash were determined in the moisture-free and fat-free bones.

RESULTS

General Health and Growth

Scours occurred in one or two calves in each group. In M 480 (Group 1) scours was evident for a total of 14 days. M 483 (Group 2) was similarly afflicted for a total of 16 days, and M 478 and M 484 (Group 3) were also afflicted for a total of 46 days. It is evident that scours was not due to magnesium carbonate.

No signs of convulsions were observed.

That M 455 and M 480 (Group 1—basal ration) and M 472 and M 483 (Group 2—basal ration and magnesium) were not normal was indicated by their tendency to stand on the fore part of their front feet. All the calves

in Group 3 (basal ration, magnesium, and vitamin D) were normal in this respect.

Growth data given in Table 1 show that from birth to 140 days of age the rate of gain in liveweight was fairly regular. After 140 days of age the rate of gain gradually decreased and was very irregular as the experiment progressed.

TABLE 1

Average rate of growth of calves fed a limited amount of whole milk and various supplements (iron, copper, magnesium, or vitamin D)

Age Days	Group 1 (Whole milk, Fe, and Cu)		Group 2 (Whole milk, Fe, Cu and Mg)		Group 3 (Whole milk, Fe, Cu, Mg and vitamin D)	
	Weight	Height	Weight	Height	Weight	Height
	lb.	*cm.*	*lb.*	*cm.*	*lb.*	*cm.*
1	96	74.49	92	77.14	94	74.06
14	115	75.23	99	77.67	114	74.59
28	123	75.77	116	76.83	121	78.10
42	141	79.58	133	79.14	135	78.51
56	160	81.48	147	81.05	150	80.64
70	179	83.18	173	84.65	168	83.59
84	196	85.09	196	86.13	186	86.13
98	210	86.56	214	89.73	205	87.83
112	230	89.10	234	89.94	219	89.10
126	263	90.57	259	92.91	230	88.56
140	273	92.28	279	94.18	257	92.71
154	280	93.34	296	95.65	280	94.38
168	289	95.45	311	97.36	298	95.88
182	304	96.72	314	98.19	308	96.92
196	316	99.06	322	99.06	334	99.99
210	336	99.69	331	100.73	340	100.96
224	355	99.89	330	103.17	355	102.23
238	358	101.60	360	102.87	373	103.07
255	357	102.87	369	103.50	382	104.97

Blood Data

The data for calcium, phosphorus, and magnesium content of the blood serum (Table 2) show that the calcium values remained fairly normal for most calves throughout the experiment. However, calves M 480 (Group 1) and M 472 and M 483 (Group 2) had blood calcium values low enough to suggest low-calcium rickets. Low-calcium rickets did not appear in Group 3.

The phosphorus content of the blood serum of all calves decreased slightly as the experiment progressed.

The serum-magnesium values for the calves in Group 1 decreased quite rapidly as the experiment continued. On the average the magnesium was below 2.0 mg. when the calves reached 120 days of age. Feeding magnesium carbonate (Groups 2 and 3) maintained the magnesium content of the blood serum at practically a normal level.

TABLE 2

Calcium, phosphorus, and magnesium in 100 cubic centimeters of blood serum of the calves in Groups 1, 2, and 3

Age Days	Calcium		Phosphorus		Magnesium	
	Range mg.	Average mg.	Range mg.	Average mg.	Range mg.	Average mg.
Group 1 (whole milk, iron, and copper)						
12– 13	11.4–11.7	11.56	8.6– 9.3	8.9	2.0–3.9	3.00
34– 45	11.0–11.8	11.29	8.6– 9.0	8.8	2.0–3.1	2.41
67– 74	10.9–12.4	11.50	6.9– 9.2	8.0	2.2–3.0	2.52
95–102	11.3–11.6	11.47	5.7– 8.6	7.2	1.9–2.6	2.25
122–136	10.0–12.4	11.28	6.2– 6.6	6.4	1.7–2.2	1.87
157–168	8.3–11.3	10.20	5.0– 6.1	5.7	1.2–1.4	1.31
172–193	9.3–11.0	10.18	5.8– 6.8	6.3	1.2–1.2	1.20
196–209	8.6–11.8	10.72	5.7– 6.2	6.0	1.1–1.6	1.46
222–234	6.9–11.1	9.57	5.9– 7.2	6.5	1.0–1.5	1.28
246–250	5.6–12.3	9.67	5.6– 6.6	6.2	1.0–1.2	1.12
Group 2 (whole milk, iron, copper and magnesium)						
12– 13	10.0–11.7	10.75	7.4– 9.7	8.6	2.7–4.4	3.37
34– 45	10.3–11.2	10.65	7.3– 8.9	8.2	2.3–2.8	2.57
67– 74	10.4–11.4	10.74	5.7–10.0	8.0	2.4–2.7	2.58
95–102	10.3–11.2	10.65	6.8– 7.5	7.2	2.6–3.7	3.12
122–136	7.9–11.6	9.83	5.9– 8.3	7.2	2.3–2.5	2.41
157–168	7.8–11.4	9.54	5.9– 9.0	7.1	2.1–2.3	2.24
172–193	8.0–10.3	9.02	6.0– 7.0	6.4	2.2–2.4	2.29
196–209	6.8–10.4	8.66	6.5– 6.9	6.6	2.2–2.3	2.28
222–234	7.3–10.8	9.07	6.9– 8.1	7.5	2.0–2.1	2.08
246–250	5.9–11.5	8.74	6.2– 8.0	6.9	1.8–2.5	2.26
Group 3 (whole milk, iron, copper, magnesium and vitamin D)						
12– 13	10.4–11.0	10.65	7.7–10.5	9.1	2.6–2.8	2.66
34– 45	10.1–10.7	10.36	5.2– 7.5	6.6	2.2–2.8	2.49
67– 74	10.6–10.8	10.70	6.4– 8.9	7.3	2.3–2.6	2.47
95–102	10.2–11.2	10.85	5.5– 8.6	7.1	2.5–3.8	2.98
122–136	10.3–10.9	10.57	6.2– 8.1	7.1	2.5–2.7	2.64
157–168	10.2–10.4	10.33	6.6– 7.8	7.2	2.4–2.9	2.59
172–193	9.6–10.5	9.92	6.1– 7.5	6.7	2.3–2.6	2.48
222–234	8.2–10.6	9.71	6.6– 8.4	7.6	2.0–2.6	2.39
246–250	9.2– 9.7	9.44	6.3– 6.8	6.6	2.3–2.5	2.37

Bone Study

The data given in Table 3 show that slight differences existed between the gross measurements of the metacarpal and metatarsal bones of Groups 1, 2, and 3. However, there was a tendency for the bones in Group 3 to be noticeably heavier and somewhat denser than the leg bones in Groups 1 and 2.

Chemical analyses of the metacarpal, metatarsal, and fifth rib bones (Table 4) show that practically no difference in ash, calcium, and phosphorus existed between Groups 1, 2, and 3. The magnesium content of the bones from the calves fed magnesium carbonate in Groups 2 and 3 was 36 per cent higher than that of the bones from the calves in Group 1 (basal ration). The bone-

TABLE 3

Average physical measurements of the left metacarpal and metatarsal bones

Calves	Green weight	Length	Smallest diameter	Width of epiphysis	Volume	Density	Breaking strength*
No.	gm.	cm.	cm.	cm.	cc.		lb.
Group 1 (whole milk, iron, and copper)							
F 549	225	21.05	2.24	5.62	175	1.28	1601
M 455	242	21.20	2.23	6.00	192	1.26	1422
M 480	273	20.90	2.51	6.12	219	1.24	1511
Av.	247	21.05	2.32	5.91	195	1.26	1511
Group 2 (whole milk, iron, copper and magnesium)							
F 543	219	22.40	2.02	5.44	166	1.31	1619
M 472	252	20.65	2.26	5.62	200	1.26	1244
M 483	254	21.35	2.42	6.10	207	1.22	1325
Av.	241	21.47	2.23	5.72	191	1.26	1396
Group 3 (whole milk, iron, copper, magnesium, and vitamin D)							
F 562	253	22.20	2.20	5.64	193	1.30	1489
M 478	262	21.10	2.35	6.00	205	1.28	1780
M 484	292	21.20	2.44	6.23	210	1.39	1565
Av.	269	21.50	2.33	5.96	203	1.32	1611

* The base fulcrums of the Tinea Olsen Breaking Machine were spaced 4 and 5/8 inches.

magnesium of the calves in Groups 2 and 3 was normal for calves of that age.

The ventral ends of the ribs from the calves in Groups 1 and 2 were enlarged; this is indicative of an insufficient supply of vitamin D. Ribs from the calves in Group 3 were normal in physical appearance (see Fig. 1).

TABLE 4

Chemical analyses of the left metacarpal, metatarsal, and fifth rib bones

Calves No.	Metacarpal and metatarsal				Fifth ribs			
	Ash pct.	Calcium pct.	Phosphorus pct.	Magnesium pct.	Ash pct.	Calcium pct.	Phosphorus pct.	Magnesium pct.
Group 1 (whole milk, iron, and copper)								
F 549	62.86	24.10	11.25	0.3595	61.18	23.51	10.80	0.3414
M 455	62.47	24.14	11.06	0.2823	60.99	23.51	10.83	0.2903
M 480	61.19	23.79	10.44	0.3194	55.19	21.15	10.19	0.2836
Av.	62.14	24.01	10.91	0.3204	59.12	22.72	10.61	0.3051
Group 2 (whole milk, iron, copper and magnesium)								
F 543	62.47	23.60	11.78	0.4935	59.82	22.31	11.42	0.4953
M 472	62.47	23.44	11.28	0.4548	59.77	22.34	11.06	0.5475
M 483	61.23	23.41	11.08	0.4776	57.19	21.40	11.19	0.4873
Av.	62.06	23.48	11.38	0.4753	58.93	22.02	11.22	0.5100
Group 3 (whole milk, iron, copper, magnesium, and vitamin D)								
F 562	64.41	24.62	11.59	0.5149	61.91	23.50	11.25	0.5510
M 478	61.87	23.51	11.70	0.4578	57.14	21.94	10.33	0.4746
M 484	62.83	24.16	11.39	0.4499	58.93	22.43	10.05	0.5150
Av.	63.04	24.09	11.56	0.4742	59.33	22.62	10.54	0.5135

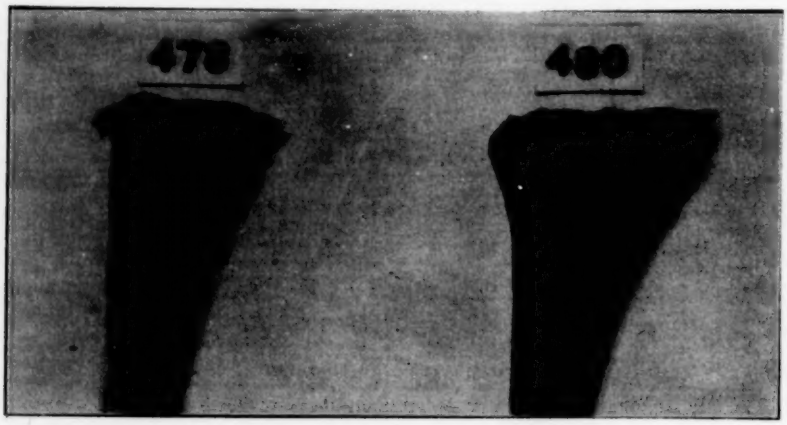

FIG. 1. Costochondral ends of two ribs. Rib 478 is from a calf in Group 3 (whole milk, iron, copper, magnesium, and vitamin D). Rib 480 is from a calf in Group 1 (whole milk, iron, and copper). Rib bones in Group 2 appeared like the ribs in Group 1.

DISCUSSION

This work confirms the observation made in 1932 (1) that low-calcium blood values may be found in calves fed a limited amount of whole milk. It is probable that serum-magnesium contents were also low at that time.

Haag and Palmer (2) have shown that a balanced condition of calcium, magnesium, and phosphorus salts in a ration for rats was essential for normal growth and function. Barbour and Winter (4), however, stress the fact that dogs fed a magnesium supplement for a prolonged period of time required an adequate intake of phosphorus in order to prevent a loss of calcium in digestion.

Judging from this work with calves, serum- and bone-magnesium values in calves fed mineralized milk do not appear to be influenced by ultraviolet radiation.

According to Duncan, Huffman, and Robinson (8) all calves would have died in convulsions if they had been kept on the milk, iron and copper ration for a much longer period of time than 255 days.

SUMMARY AND CONCLUSION

Three calves fed a basal ration containing a limited amount of winter milk plus iron and copper salts were compared with three calves fed a like ration supplemented with magnesium carbonate and with three calves fed the basal ration supplemented with magnesium carbonate and vitamin D (ultraviolet light). When magnesium was not added, blood serum-magnesium and bone-magnesium were lowered. When magnesium was added,

the blood serum-magnesium and bone-magnesium were normal. Ultra-violet radiation produced somewhat heavier bones (green weight, in those calves fed the milk-iron-copper-magnesium ration.

ACKNOWLEDGMENT

The authors are indebted to the General Electric Company, Nela Park, Cleveland, Ohio, for supplying and periodically checking the S_1 Sunlamps.

REFERENCES

(1) KNOOP, C. E., KRAUSS, W. E., AND WASHBURN, R. G. The development of nutritional anemia in dairy calves. J. DAIRY SC. 18: 337–347. 1935.

(2) HAAG, J. R., AND PALMER, L. S. The effect of variations in the proportions of calcium, magnesium, and phosphorus contained in the rat. J. Biol. Chem. 76: 367–389. 1928.

(3) ELMSLIE, W. P., AND STEENBOCK, H. Calcium and magnesium relations in the animal. J. Biol. Chem. 82: 611–632. 1929.

(4) BARBOUR, H. G., AND WINTER, J. E. Magnesium absorption in dogs and its effect upon the metabolism of calcium. J. Pharm. Exp. Ther. 43: 607–620. 1931.

(5) KRUSE, H. D., ORENT, E. R., AND MCCOLLUM, E. V. Studies on magnesium deficiency in animals. III. Chemical changes in the blood following magnesium deprivation. J. Biol. Chem. 100: 603–643. 1933.

(6) CUNNINGHAM, I. J. Magnesium in animal diets. The influence of the level of dietary magnesium on the magnesium and calcium contents of the bones, the bodies, and the blood serum of rats. New Zealand J. Sc. and Tech, 15: 191–198. 1933.

(7) TUFTS, E. V., AND GREENBURG, D. M. The biochemistry of magnesium deficiency. II. The minimum magnesium requirement for growth, gestation, and lactation, and the effect of the dietary calcium level thereon. J. Biol. Chem. 122: 715–726. 1938.

(8) DUNCAN, C. W., HUFFMAN, C. F., AND ROBINSON, C. S. Magnesium studies in calves. I. Tetany produced by a ration of milk or milk with various supplements. J. Biol. Chem. 108: 35–44. 1935.

(9) HUFFMAN, C. F., AND DUNCAN, C. W. Vitamin D studies in cattle. II. The vitamin D sparing action of magnesium in the ration of dairy cattle. J. DAIRY SC. 18: 605–620. 1935.

(10) CLARK, E. P., AND COLLIP, J. B. A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem. 63: 461–464. 1925.

(11) BELL, R. D., DOISY, E. A., AND BRIGGS, A. P. Determination of inorganic phosphorus, of hydrolysable, and total acid soluble phosphorus in blood, plasma, or serum. Mathews Physiological Chemistry, 4th Edition, pp. 1083. 1925.

(12) DENIS, W. Determination of magnesium in blood, plasma, and serum. Mathews Physiological Chemistry, 4th Edition, pp. 1078. 1925.

(13) WASHBURN, M. L., AND SHEAR, M. J. Composition of bone. XIII. Direct gravimetric determination of calcium, magnesium, and phosphate. Proc. Soc. Exp. Biol. and Med. 29: 625–627. 1932.

(14) FREAR, D. E. H., AND KAHLENBERG, O. J. A study of the accuracy of the McCudden method for calcium and magnesium in biological materials. J. Biol. Chem. 100: 85–95. 1933.

(15) SCOTT, W. W. Determination of phosphorus as magnesium pyrophosphate. Standard Methods of Chemical Analysis, pp. 312–316. 1917.

American Dairy Science Association Announcements

ANNUAL MEETING, JUNE 27–30, 1939

Pullman, Washington, and Moscow, Idaho

SPECIAL CONVENTION CAR

Members planning to travel to the annual convention by train are advised that the Northern Pacific Railway is planning definitely to provide, for the benefit of the convention party, special cars to be attached to the famous North Coast Limited, leaving Chicago on June 22. See the advertisement of this line in the Journal and note the three plans presented by them for convention travelers and described in detail in an illustrated folder, which may be obtained from the association's Secretary-Treasurer, R. B. Stoltz, of Ohio State University, Columbus, Ohio. The Northern Pacific is known as the Yellowstone Park Line and follows a very scenic route to the convention.

HOUSING

New modern dormitories will be available at both the State College of Washington and at the University of Idaho for use of the members during the convention. The rates will be $1.25 per person per night, for one or two nights. For three nights or more the rate will be $1.00 per person per night. The rate for children will be the same as for adults. The dormitories are close to the meeting places. Rooms will also be available at hotels in both Moscow and Pullman. Reservation of rooms may be made by writing to Professor J. C. Knott, State College of Washington, or Dr. A. O. Shaw, University of Idaho, Moscow, Idaho.

REGISTRATION

Members may register either at the University of Idaho or the State College of Washington, beginning June 26, 1939.

LAST CALL FOR PAPERS

Titles and abstracts of papers must be in the hands of the program committee not later than April 15. Address: Vice-Dean E. V. Ellington, Department of Dairy Husbandry, State College of Washington, Pullman, Washington.

NEW MEMBERS FOR 1939—BY STATES

APPLICATIONS RECEIVED BETWEEN JANUARY 11 AND MARCH 6, 1939

ARKANSAS

Gregg, V. L., Chairman, State Membership Committee. (Total to date 1.)
Smith, O. D., Conway, Ark.

CALIFORNIA

Roadhouse, C. L., Chairman, State Membership Committee. (Total to date 9.)
Abbot, Fred H., Davis, Calif.
Beard, William J., Upland, Calif.
Cordray, Donald A., Berkeley, Calif.
Herzog, Max L., Petaluma, Calif.

CONNECTICUT

Mann, A. I., Chairman, State Membership Committee. (Total to date 1.)
Wilbur, Willis, Hartford, Conn. (Student Affiliate) (nominated by Anderson).

DISTRICT OF COLUMBIA

LeFevre, P. E., Chairman, State Membership Committee. (Total to date 2.)

GEORGIA

Bennett, F. W., Chairman, State Membership Committee. (Total to date 5.)
Frey, E. W., Douglas, Ga.
Harvey, Jas. J., Atlanta, Ga.
Smith, D. M., Atlanta, Ga.
Martorell, Miguel, Athens, Ga. (Stud. Aff.).

ILLINOIS

Kuhlman, A. F., Chairman, State Membership Committee. (Total to date 4.)
Synold, Richard E., Chicago, Ill.
Wright, J. Harold, Greenville, Ill.

INDIANA

Wilbur, J. W., Chairman, State Membership Committee. (Total to date 26.)
Alden, E. G., Terre Haute, Ind.
Andersen, J. S., Gary, Ind.
Bender, John E., Terre Haute, Ind.
Buzolits, John A., South Bend, Ind.
Dugdale, R. K., Indianapolis, Ind.
Foulkes, Harold, Kentland, Ind.
Hedges, Byron D., Terre Haute, Ind.
Holt, Arthur P., Indianapolis, Ind.
Kerr, R. C., Evansville, Ind.
Krannert, H. C., New Augusta, Ind.
Palmer, James L., South Bend, Ind. (by Parfitt).
Risch, Lawrence N., Vincennes, Ind.
Schacht, W. F., Huntington, Ind.
Scharf, Irvin C., New Albany, Ind.
Suhre, F. H., Columbus, Ind.

Wren, D. R., Chicago, Ill. (by Parfitt).
Mather, Robert, West Lafayette, Ind. (Stud. Aff.).
Myers, Max E., Bourbon, Ind. (Stud. Aff.) (by Parfitt).

IOWA

Johnston, Floyd, Chairman, State Membership Committee. (Total to date 4.)
Hansen, Elmer N., Ames, Iowa.
Mennen, K. E., Des Moines, Iowa.

KANSAS

Linn, J. W., Chairman, State Membership Committee. (Total to date 1.)

LOUISIANA

Lush, R. H., Chairman, State Membership Committee. (Total to date 1.)
Gelpi, A. J., Jr., University, La.

MARYLAND

England, C. W., Chairman, State Membership Committee. (Total to date 7.)
Marty, Ivan M., Cockeysville, Md.
McElwee, H. W., Hagerstown, Md.
Butler, Mason, Dickerson, Md. (Stud. Aff.).
Remsberg, LeRoy, Baltimore, Md. (Stud. Aff.).

MASSACHUSETTS

Frandsen, J. H., Chairman, State Membership Committee. (Total to date 4.)
Keyock, Nicholas L., Amherst, Mass. (Stud. Aff.).
Lipman, Leo O., Springfield, Mass. (Stud. Aff.).
Rodda, Charles, Jr., Springfield, Mass. (Stud. Aff.).
Widland, Myron A., Amherst, Mass. (Stud. Aff.).

MICHIGAN

Weaver, Earl, Chairman, State Membership Committee. (Total to date 29.)
Beal, Victor C., Stanton, Mich.
Haradine, Carl E., Kalamazoo, Mich.
Miller, Charles D., Eaton Rapids, Mich.
Roth, Armin A., Hastings, Mich.
Bryan, Paul S., East Lansing, Mich. (Stud. Aff.).
Buckingham, George E., East Lansing, Mich. (Stud. Aff.).
Buth, Martin, Jr., East Lansing, Mich. (Stud. Aff.).
Campan, Francis, East Lansing, Mich. (Stud. Aff.).
Ely, Ray Edwin, East Lansing, Mich. (Stud. Aff.).
Gilbert, Bradley L., East Lansing, Mich. (Stud. Aff.).
Hale, E. B., East Lansing, Mich. (Stud. Aff.).
Hankinson, Edwin M., East Lansing, Mich. (Stud. Aff.).
Snyder, Woodrow, East Lansing, Mich. (Stud. Aff.).
Stephenson, Robert, East Lansing, Mich. (Stud. Aff.).

MINNESOTA

Macy, H., Chairman, State Membership Committee. (Total to date 17.)
Barker, E. M., Rochester, Minn.
Boney, Richard, St. Paul, Minn.

Carithers, R. L., Murfreesboro, Tenn.
Chapman, K. W., Hutchinson, Minn.
Duncan, James F., St. Paul, Minn.
Fountaine, F. C., St. Paul, Minn.
Gibson, Douglas L., St. Paul, Minn.
Hawkins, Orville J., St. Paul, Minn.
Jordan, P. S., Morris, Minn.
Luby, Daniel J., St. Paul, Minn.
Naylor, H. Brooks, Ithaca, N. Y.
Ratzlaff, Alfred, St. Paul, Minn.
Richardson, J. W., Manitowoc, Wis.
Sorensen, C. M., Dubuque, Iowa
Ward, Harlan, Minneapolis, Minn.
Wolberg, E. B., Wayzata, Minn.

MISSISSIPPI

Herzer, F. H., Chairman, State Membership Committee. (Total to date 2.)

MISSOURI

Herman, H. A., Chairman, State Membership Committee. (Total to date 3.)
Fear, E. D., Kansas City, Mo. (by Ragsdale).
Hughes, R. D., Becker, Mo.

MONTANA

Nelson, J. A., Chairman, State Membership Committee. (Total to date 6.)
Boyd, John C., Kalispell, Mont.
Heath, C. L., Billings, Mont.
McIver, Kenneth, Great Falls, Mont.

NEW YORK

Salisbury, G. W., Chairman, State Membership Committee. (Total to date 11.)
Browning, R. A., Buffalo, N. Y.
Elliott, F. Irvine, Ithaca, N. Y.
Fuller, Harold B., Norwich, N. Y. (by Guthrie).
Gilmore, Dr. L. N., Buffalo, N. Y.
Willett, E. L., Ithaca, N. Y.

OHIO

Salisbury, S. M., Chairman, State Membership Committee. (Total to date 28.)
Deubler, Dr. E. C., Newtown, Pa. (by Sutton).
Alleshouse, Paul R., Baltic, Ohio (Stud. Aff.) (by Stoltz).
Bell, Henry, Peninsula, Ohio (Stud. Aff.) (by Stoltz).
Burkey, Lloyd C., Columbus, Ohio (Stud. Aff.) (by Stoltz).
Cleveland, G. Eugene, Galena, Ohio (Stud. Aff.) (by Stoltz).
Eisenhut, Emil, Carrollton, Ohio (Stud. Aff.) (by Stoltz).
Gillespie, Dale, Columbus, Ohio (Stud. Aff.) (by Stoltz).
Kesselring, Lowell M., Columbus, Ohio (Stud. Aff.) (by Stoltz).
Krogg, Vernon C., Lakewood, Ohio (Stud. Aff.) (by Stoltz).
Mally, Elliott C., Columbus, Ohio (Stud. Aff.) (by Stoltz).
Meeks, Benton, Lakewood, Ohio (Stud. Aff.) (by Stoltz).
Moody, David B., Cleveland, Ohio (Stud. Aff.) (by Stoltz).
Pallas, George E., Elyria, Ohio (Stud. Aff.) (by Stoltz).

Shook, L. C., Columbus, Ohio (Stud. Aff.) (by Stoltz).
Strait, Howard, Delaware, Ohio (Stud. Aff.) (by Stoltz).
Thomas, Harold C., Columbus, Ohio (Stud. Aff.) (by Stoltz).
Woodyard, Wayne, Columbus, Ohio (Stud. Aff.) (by Stoltz).

OREGON

Wilster, G. H., Chairman, State Membership Committee. (Total to date 1.)
Stout, Roy E., Corvallis, Ore. (Stud. Aff.).

PENNSYLVANIA

Dahle, C. D., Chairman, State Membership Committee. (Total to date 23.)
Adams, James, State College, Pa.
Arthur, Richard R., Waynesboro, Pa.
Drummond, W. Robert, Ridgewood, N. J.
Durand, Alfred M., Chambersburg, Pa.
Josephson, D. V., State College, Pa.
Keeney, Mark H., Cedar Grove, N. J.
Lawhorn, R. K., State College, Pa.
Myers, Claude H., Pennsylvania Furnace, Pa.
McLearen, C. S., State College, Pa.
Pence, John T., Reading, Pa.
Perry, Ernest A., Philadelphia, Pa.
Perry, N. A., State College, Pa.
Preston, Forest, Providence, R. I.
Rivers, Philip W., State College, Pa.
Shortlidge, F. T., Upper Darby, Pa.
Warren, F. G., State College, Pa.
Wood, Jas. L., Jr., Red Hill, Pa.
Bigott, Gus, State College, Pa. (Stud. Aff.).
DeTurk, Lester E., State College, Pa. (Stud. Aff.).
Ellis, N. D., State College, Pa. (Stud. Aff.).
Miller, Graham M., Bethlehem, Pa. (Stud. Aff.).
Moss, Clarence M., State College, Pa. (Stud. Aff.).
Sorensen, Howard C., State College, Pa. (Stud. Aff.).

SOUTH CAROLINA

LaMaster, J. P., Chairman, State Membership Committee. (Total to date 1.)

UTAH

Morris, A. J., Chairman, State Membership Committee. (Total to date 11.)
Anderson, H. D., Ephraim, Utah.
Calder, David H., Vernal, Utah.
Fenton, R. L., Parowan, Utah.
Johnson, Max, Preston, Idaho.
Olsen, O. S., Provo, Utah.
Pittman, Robert G., Wellsville, Utah.
Roskelley, Lowell, Ogden, Utah.
Warr, M. J., Beaver, Utah.
Cox, Chad, Fairview, Utah (Stud. Aff.).
Ogden, D. W., Logan, Utah (Stud. Aff.).

VERMONT

Ellenberger, H. B., Chairman, State Membership Committee. (Total to date 6.)
Weatherby, Edwin J., Burlington, Vt.
Dana, Richard E., Burlington, Vt. (Stud. Aff.).
Livak, Chas., Burlington, Vt. (Stud. Aff.).
Pitman, George, Burlington, Vt. (Stud. Aff.).
Plumb, Robert E., Burlington, Vt. (Stud. Aff.).
Young, Robert M., Burlington, Vt. (Stud. Aff.).

VIRGINIA

Connelly, R. G., Chairman, State Membership Committee. (Total to date 4.)
Fisher, R. B., Richmond, Va.
Luck, Beattie C., Richmond, Va.
Pfalgraf, Rene D., Hollins, Va.
Renshaw, J. Ralph, Big Stone Gap, Va.

WASHINGTON

Bendixen, H. A., Chairman, State Membership Committee. (Total to date 15.)
Courtney, Daniel S., Shelton, Wash.
Drake, John B., Wenatchee, Wash.
Holland, A. W., Chehalis, Wash.
Jones, Davis, Yakima, Wash.
Krassin, C. W., Burlington, Wash.
Kristensen, A., Seattle, Wash.
Larson, Harold, Seattle, Wash.
Miller, Ray, Seattle, Wash.
Olsen, John E., Shelton, Wash.
Palmer, W. Horace, Steilacoom, Wash. (by Hill).
Pierce, C. E., Olympia, Wash.
Poston, Cecil A., Spokane, Wash.
Tucker, R. D., Pullman, Wash.
Wertman, Albert P., Ft. Steilacoom, Wash.
Morgan, Max, Pullman, Wash. (Stud. Aff.).

WISCONSIN

Weckel, K. G., Chairman, State Membership Committee. (Total to date 16.)
Anderegg, Tracy, Portland, Ore.
Bly, James A., Madison, Wis.
Curless, Robert, Madison, Wis.
Galles, Leo T., Port Washington, Wis.
Goergen, John C., Shawano, Wis.
Harris, Robert J., Janesville, Wis.
Ireland, Wm. R., Racine, Wis.
Kaeser, W. G., Madison, Wis.
Krause, F. W., Waukesha, Wis.
Lucia, Floyd B., Eau Claire, Wis.
Marty, Carl O., Jr., Monroe, Wis.
Montemurro, Frank, Kenosha, Wis.
Stein, Bruno, Madison, Wis.
Thew, Harvey E., Madison, Wis.

Wilke, Theodore M., Milwaukee, Wis.
Zurheide, A. W., Sheboygan, Wis.

WYOMING

Willard, H. S., Chairman, State Membership Committee. (Total to date 1.)
Steward, LaVere, Thermopolis, Wyo.

CANADA

BRITISH COLUMBIA

Rive, Henry, Chairman, State Membership Committee. (Total to date 4.)
Christie, C. E., Ocean Falls, B. C.
Crabtree, Geo. H., Abbotsford, B. C.
Moore, P. H., Essondale, B. C.
Wasson, F. C., Kelowna, B. C.

CANADA

EASTERN PROVINCES

Raithby, G. E., Chairman, State Membership Committee. (Total to date 2.)
Malcolm, Calir Keith, Toronto, Ont. (by Thornton).
Singleton, John Franklin, Ottawa, Ont. (by Sproule).

MISCELLANEOUS

Bracke, George M., Pittsburgh, Pa.
Phillips, Paul H., Madison, Wis.
Reed, A. J., Lovelock, Nev.

VOLUME XXII APRIL, 1939 NUMBER 4

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ
Ohio State University, Columbus, Ohio, Sec.-Treas.

ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
R. C. HIBBEN, 1105 Barr Bldg., Washington, D. C., Exec. Sec.

INTERNATIONAL ASSOCIATION OF MILK DEALERS
R. E. LITTLE, 309 W. Jackson Blvd., Chicago, Illinois, Exec. Sec.

Editorial Committee

H. H. SOMMER, Madison, Wisconsin, A. D. S. A.
J. C. HENING, Geneva, New York, A. D. S. A.
HAROLD PRATT, Philadelphia, Pennsylvania, I. A. I. C. M.
G. D. TURNBOW, Oakland, California, I. A. I. C. M.
W. D. DOTTERRER, Chicago, Illinois, I. A. M. D.
A. J. POWERS, Brooklyn, New York, I. A. M. D.

CONTENTS

Bacteriology
Breeding
Butter
Cheese
Chemistry
Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
Physiology

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Fay, A. C., Holm, G. E., Marquardt, J. C., and Tracy, P. H.
Dahlberg, A. C.

ABSTRACTORS

Allen, N. N.
Archibald, J. G.
Atkeson, F. W.

Babcock, C. J.
Baltzer, A. C.
Barkman, J. O.
Bartlett, J. W.
Becker, R. B.
Bendixen, H. A.
Bennett, F. W.
Bird, E. W.
Burgwald, L. J.
Burri, R.
Brueckner, H. J.
Burke, A. D.
Bushnell, L. D.

Cannon, C. Y.
Carpenter, D. C.
Cave, H. W.
Clevenger, W. L.
Cole, W. C.
Copeland, L.
Coulter, S. T.
Cunningham, O. C.
Cunningham, W. S.

Dahlberg, A. C.
Darnell, A. L.
Demeter, K. J.
Doan, F. J.
Dorsey, L. M.
Downs, P. A.

England, C. W.
Erb, J. H.
Espe, D. L.

Fabian, F. W.
Frayer, J. M.
Frazier, W. C.
Fuller, J. M.

Geipi, A. J.
Golding, N. S.
Goss, E. F.
Greenbank, G. R.
Gullickson, T. W.
Guthrie, E. S.

Hansen, Arne
Hening, J. C.
Herrington, B. L.
Herser, F. H.
Holdaway, C. W.
Horrall, B. E.
Huffman, C. F.

Jacobson, C. O.
Jensen, Chris
Josephson, D. V.

Keith, J. I.
Knight, D.
Krauss, W. E.

LaMaster, J. P.
Leighton, A.
Lindquist, H. G.
Locke, R. B.
Lucas, P. S.
Lush, J. L.

Mack, M. J.
Macy, H.
Mann, A. I.
Marquardt, J. C.
Martin, W. H.
Mead, S. W.
Moore, L. A.
Morris, A. J.
Mueller, W. S.

Nelson, D. H.
Nelson, J. A.

Overman, O. R.

Palmer, C. C.
Parfitt, E. H.
Petersen, W. E.
Price, W. V.

Reid, W. H. E.
Richardson, G. A.
Riddell, W. H.

Schultz, E. N.
Sommer, H. H.
Stark, C. N.
Swope, W. D.

Tarassuk, N. P.
Theophilus, D. R.
Thomsen, L. C.
Thurston, L. M.
Totman, C. C.
Trout, G. M.
Tuckey, S. L.

Webb, B. H.
Weckel, K. G.
White, G. C.
Wilbur, J. W.
Willater, G.
Wylie, C. E.

Yale, M. W.

JOURNALS

American Creamery and Poultry Produce Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics

Biochemical Journal
Biochemische Zeitschrift

Canadian Dairy and Ice Cream Journal
Certified Milk
Cornell Veterinarian

Dairy Industries
Deutsche Molkerei Zeitung

Endocrinology

Food Industries
Food Manufacture
Food Research

Guernsey Breeders Journal
Ice and Refrigeration
Ice Cream Field
Ice Cream Industry
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Jersey Bulletin
Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Chemical Society
Journal of American Veterinary Medicine Association
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Experimental Medicine
Journal of General Physiology
Journal of Heredity
Journal of Genetics
Journal of Infectious Diseases
Journal of London Chemical Society
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology

Kaseindustrie
Kolloid-Zeitschrift

Lancet
Le Lait

Milchwirtschaftliche Forschungen
Milchwirtschaftliche Zeitung
Milk Dealer
Milk Industry
Milk Plant Monthly
Molkerei Zeitung

National Butter and Cheese Journal

Pacific Dairy Review
Proceedings of Society of Animal Production
Proceedings of Society of Experimental Biology and Medicine

Tierernährung
Tierzüchter
Trudy Vologodskogo Molochnogo Institut

Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Zeitschrift für Physikalische Chemie, Abt. A and B
Zeitschrift für Untersuchung der Lebensmittel
Zeitschrift für Züchtung. Reihe B. Tierzuchtung und Züchtungbiologie
Zentralblatt für Bacteriologie
Züchtungskunde

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BUTTER

140. Some Vital Points in the Butter Industry Figured in Formulas. E. VAILLANT. Le Lait *18:* 179, 897–919, 1938.

Formulas are given for calculating the value of butter based on a series of factors, among which are the butter fat content of the milk or cream from which the butter was made, and the by-products remaining after the buttermaking operation. A.H.J.

CHEESE

141. An Apparatus for Measuring the Elastic and Plastic Properties of Cheese Curd. G. W. SCOTT BLAIR, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res. *9:* 347–350. 1938.

An apparatus is described for measuring the elastic and plastic deformations of cheese curd under a compressive load at the time it is ready for cutting. The apparatus is considered to be more desirable than those which simply determine the force required to cut the curd since the results can be expressed in absolute (C.G.S.) units. The recoverable (elastic) deformation was found to be quite large in relation to the non-recoverable deformation. The elastic deformation is inversely proportional to shear modulus and is believed to be the best single criterion of "firmness" in deciding the optimum time to cut the curd. S.T.C.

142. The Composition of Cheese Rinds. W. L. DAVIES, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res. *9:* 342–346. 1938.

Rinds from ten samples of English, three Canadian, and five New Zealand Cheddar cheese were collected and analyzed. The rinds from three Stilton cheese were also analyzed for comparison. Rinds from aged Cheddar cheese were found to contain 15 to 20 per cent of moisture, and those from unripe cheese up to 27 per cent moisture. Stilton rinds contained an average of 17 per cent moisture. On a dry matter basis, Cheddar rinds contained less salt than the inside of the cheese. Due to increased salt concentration in the water in the outer layers there is a tendency for the salt to diffuse slowly into the inner layers of higher moisture content. There is an appreciable loss of fat from the Cheddar rinds through absorption by the bandage. The rinds were found to have a higher crude protein content, a higher calcium and phosphorus content and a higher Ca/P ratio than the interior of the cheese. S.T.C.

143. The Solubility of Cheddar Cheese Curd in Sodium Chloride Solutions. F. H. McDowall and L. A. Whelan. Dairy Research Institute (N.Z.), Palmerston North, New Zealand. J. Dairy Res. *9:* 339–341. 1938.

Measurements are reported of the solubility, in water and in sodium chloride solutions of different concentrations, of the protein of cheese at various times after manufacture. The solubility in 3 to 10 per cent sodium chloride solutions reached 100 per cent within 7 days after manufacture of the cheese, and remained at that value throughout the life of the cheese (8 months). S.T.C.

CHEMISTRY

144. The Solubility of Casein in Salts of Certain Organic Acids and Its Fractionation by Means of These Acids. R. H. McKee, Columbia Univ. and S. P. Gould, Bureau of Dairy Industry. J. Agr. Res., *57:* 125–143, July, 1938.

Solutions of sodium cymenesulphonate, potassium thiocyanate and sodium benzenesulphonate of 5 and 7.5%, 2.5 and 5%, and 5 and 10% concentrations, respectively, were used as solvents for acid-precipitated casein purified by washing with acidulated water, pure water, alcohol and finally with ether. Solubility of casein was relatively high, and increased in each case with the increased concentration of the solvent, and also with increased solution temperatures which were 15° C. (59° F.), 30° C. (86° F.) and 45° C. (113° F.). The increased solubility with increasing concentrations of solutions is considered to be one proof of the non-homogeneity of casein.

Fractionation studies with the casein mentioned, and with grain curd casein and Hammarsten casein where tried, showed that soluble and insoluble fractions could be produced at 45° C. (113° F.) by 5 and 10% sodium benzenesulphonate solution and by 5% sodium cymenesulphonate. The soluble and insoluble fractions varied in their phosphorus and nitrogen contents, phosphorus and nitrogen ratio, formaldehyde absorbing ability and in their solubility in pyridine. L.M.T.

145. A Precise Method for the Determination of Carotene in Forage. Donald W. Bolin and Assad M. Khalapur, Univ. of Idaho, Moscow, Idaho. J. Ind. & Eng. Chem., Analyt. Ed. *10:* 8, 417–18, 1938.

Peterson and Hughes' modification of Guilbert's method for extracting carotene from forage has been further modified by the authors to permit the use of definite quantities of reagents and to avoid the formation of emulsions. More carotene is recovered by the modified method and more precise results obtained. B.H.W.

146. Analysis of Sugar Mixtures Containing Dextrose, Levulose, Maltose, and Lactose. F. W. ZERBAN AND LOUIS SATTLER, N. Y. Sugar Trade Lab., N. Y., J. Ind. & Eng. Chem., Analyt. Ed. *10:* 12, 669–74, 1938.

In the proposed method the total reducing sugars are determined by means of Fehling solution, the monosaccharides with Steinhoff's modification of Barfoed's reagent, and levulose by the method of Jackson and Mathews. Lactose is found by oxidation to mucic acid or preferably by copper reduction after fermenting off the other sugars. Four equations are thus obtained from which the percentage of each sugar can be calculated. It has been found that both maltose and lactose have a slight reducing effect on Steinhoff's reagent as well as on Jackson and Mathews' reagent, and it is necessary to apply corresponding corrections. The quantities of dextrose, levulose and lactose found in known mixtures agree well with those taken, but the result for the maltose is less reliable because it is obtained by difference. B.H.W.

147. The Isolation of a Flavoprotein from Cow's Milk. H. S. CORRAN AND D. E. GREEN, Dept. of Biochem., Cambridge, Mass. Biochem. J. *32:* 2231, 1938.

It was observed that concentrated solutions of the xanthine oxidase of milk were characterized by a pronounced orange coloration. A flavoprotein was isolated which accounted for some, though not all of this color. There are, very likely, other flavoprotein compounds in milk.

In concentrated solutions the flavoprotein is orange-red in color; dilute solutions appear brownish yellow. On addition of hyposulphite to a neutral solution of the protein, the color is bleached, and restored on shaking with air. Reoxidation is instantaneous when the hyposulphite is oxidized to sulphate.

There are three main absorption bands, with maxima at 279, 350, and 450 mu respectively. The isolation and properties of a flavoprotein and its prosthetic derivative from milk are described. K.G.W.

148. The Colorimetric Determination of pH in Milk and Whey by Means of the "Wulff" pH Tester. R. ASCHAFFENBURG, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res. *9:* 336–338. 1938.

The pH of whey determined colorimetrically by means of the "Wulff" pH tester was found to agree within about 0.1 pH unit to the values secured potentiometrically using a glass electrode. Slightly inferior results were obtained with milk. S.T.C.

DISEASE

149. Die Frischmilchschnellagglutination zum Nachweis der Abortus-Bang-Infection. (The Use of the Fresh-milk-rapid-agglutination Test for the Diagnosis of Abortus-Bang-Infection.) DR. EHRLICH. Zschr. Infektkr. Haustiere. *52:* (⅘), 163–179.

Since attempts are now being made to control infectious abortion intensive investigations are under way to improve methods of diagnosis and to detect carriers. One of the methods suggested is to examine the milk of cows by means of the agglutination test.

The test is made on fat-free milk or on whey. The milk is diluted about ten to forty times and mixed with an equal amount of either stained or unstained abortus antigen on a glass plate and incubated for 5 minutes at room temperature. Agglutination in 10 and 20 dilutions are considered as positive.

A study was made using the rapid milk agglutination test on 1044 samples of milk and the results compared to those on the blood serum of these same animals, using the tube agglutination test. There was agreement in 73.9%, disagreement in 16.3% and doubtful tests in 9.6%. For this reason this method cannot be recommended in place of the agglutination test using blood serum L.D.B.

150. Die Wirkung des Volkmarverfahrens und der Sufrogelbehandlung auf die Rinderbrucellose. (The Treatment of Bovine Brucillosis by the Volkmar Method and by the Use of Certain Sulphur Compounds.) H. ZELLER UND W. STOCKMAYER. Zschr. Infektkr. Haustiere. *50:* ⅓, 165–179.

Some writers believe that this disease may be influenced unfavorably by certain mineral deficiencies in the soil and the usual stock-feeds. By supplying these deficiencies artifically it was believed that the constitution of the animal might be so improved as to protect it from the disease. Volkmar considered it possible to use the injection method of introducing these mineral solutions. In this study sulphur compounds were employed in comparison with other mineral mixtures.

The sulphur compound mostly studied consisted of a sterile suspension of 0.3% of finely divided sulphur in gelatin, prepared commercially in Germany.

The experimental animals were selected on the basis of the agglutination and complement-fixation tests and a clinically sound udder. The tests were conducted on 18 pregnant heifers. Six were given the Volkmar treatment, six the sulphur suspension and six held as untreated controls. The first two groups were treated for 13 days and then given 100 to 200 cc. of a Brucella culture in 900 or 800 cc. of physiological salt solution. This was a pooled culture of 10 freshly isolated strains, diluted so that each cc. con-

tained approximately 72 million organisms. The culture was introduced into the stomach by means of a tube through the nostril. Treatments were again continued until the 24th day and culture given again. Treatment was then continued until the 78th day.

Of the Volkmar group 5 animals aborted, and the sixth calf died of pneumonia caused by the abortion organism. In the sulphur treated group five likewise aborted and one calf died of penumonia. All controls aborted and all the fetuses carried the organisms. The organisms were found in the milk of all these animals. In the Volkmar group two animals developed mastitis, as compared to three in the sulphur group and five in the control.

Antibody formation was not retarded by the mineral treatments, and in fact, the appearance of antibodies in the blood was considerably accelerated.

These results are contrary to those reported earlier on naturally infected animals treated by the Volkmar method and with the sulphur compounds above described, since early reports had been very favorable for such treatment. L.D.B.

151. Kulturelle und experimentelle Untersuchungen über die Aetiologie der Aktinomykose des Rindes. (Cultural and Experimental Studies on the Etiology of Actinomyces in Cattle.) HANS SEDLMEIER. Zschr. Infektkr. Haustiere. *50:* ⅗, 129–164.

Under the term Actinomycosis, or ray-fungus disease, are included those chronic processes which result in formation of yellowish granular pus and granulation tissue containing the ray fungus. Cattle are the most commonly infected of all domesticated animals. The disease may involve any organ but is most common in the mouth, tongue, and udder.

A variety of organisms come into question in those lesions containing pus, so that various cultural methods come into use in determining the etiology and in many instances the transmission to experimental animals is necessary. Another difficulty which is encountered in this work is that in early studies improper experimental animals were selected for study, and the fact that such a variety of names was used to describe it. With the introduction of the microscope a greater degree of accuracy was obtained.

Cultures were isolated from 59 cases of actinomyces. These were obtained from 34 infections of the tongue, 25 from soft tissue and bones, 27 cultures from the first, and 21 from the last group.

In all cases from the bones *Streptothrix israeli* was isolated, from the 27 cases of actinomycosis of the mouth parts and tongue the *Actinobacillus lignieresii* was obtained. Neither the *Bacterium pyogenes* or *Staphylococcus pyogenes* were found. Mixed infections of *Botryomyces* and *Actinomyces* were not found in the same glands as has been reported in swine.

Laboratory methods for the isolation and identification of the different organisms are given. The Actinobacillus colonies were very much alike,

and the organisms were Gram-negative, short, thin rods. The growth of the Streptothrix was more vigorous, and the colonies were more dry and wrinkled. Bouillon was not clouded by either of the above organisms. The Streptothrix was usually a Gram-positive rod with thickened ends and V and L shaped forms. On glycerinated potato media longer irregular threads were formed.

Both these organisms produced tubercle-like lesions in the peritoneum of rabbits and guinea pigs.

The Actinobacillus produced a more extensive fibrinous and exudative type of peritonitis, with small multiple abscesses in the liver and lungs the size of a pea. The action of the Streptothrix in the peritoneum was less severe, and in the lung there was more of a lobular type of pneumonia.

Animals injected with the Actinobacillus became somewhat allergic to mallein but not to the extracts of the Actinobacillus when grown on glycerine broth. These organisms did not appear to produce exo-toxins. A very extensive description of the organisms and their pathological changes is included. They were negative to tuberculin. Animals treated with the Actinobacillus produced sera which were weakly positive for the glandular organisms by the complement-fixation and agglutination test.

L.D.B.

152. Zur Frage der Allergen-Auswertung. (The Question of Allergic Evaluation.) DR. K. BELLER. Zschr. Infektkr. Haustiere. *50*: 4, 336–344.

A comparison was made between tuberculin, brucellin, and mallein on 120 cattle to determine specificity of the reactions. The products were injected in 0.1 cc. doses intracutaneously and reactions recorded for seven days. A skin thickening of 3 mm. or more was considered a positive reaction; 1.5 mm. a doubtful reaction.

Cattle were selected rather than guinea pigs since they are less sensitive to allergic reactions than the latter. The skin of the guinea pig may be increased in thickness as much as 10 times, while the skin of the bovine is usually only doubled in positive reactions.

Of the cattle tested 72.5 per cent reacted to tuberculin; 11.7 per cent to brucellin; 30.0 per cent to mallein. The reactions between mallein and tuberculin were parallel in all but 3 animals, and between brucellin and tuberculin in all but 5 animals.

These cross reactions are discussed in their relation to the tuberculin test, and it was suggested that certain of the "no lesion reactors" may be due to non-specific allergic response to other infections.

In general the reactions which developed for mallein and brucellin were about parallel from day to day but not usually parellel with those produced by tuberculin. In all instances the tuberculin reaction was more pronounced

and usually reached its peak after the other reactions had begun to subside. The most pronounced reaction for brucellin and mallein was on the first or second day following the injection. For tuberculin, the reaction became most pronounced between the third and seventh day. L.D.B.

153. Beitrag zur Technik der abfestuften Rohrchenagglutination. (Report on the Evaluation of the Tube Agglutination Test.) ALFRED PERZ. Zschr. Infektkr. Haustiere. *50:* 4, 313–336.

This report consists largely of a discussion of the results obtained by various persons in reading tube agglutination tests for Bang's disease. A series of tests were made and the results read by different persons. The results were then evaluated on the basis of probable error determinations.

Three methods were studied.

1. The so-called single measurement method in which various amounts of diluted serum are added to 1 cc. of antigen.

2. The chain method of dilution in which a certain amount of serum is added to the first tube of 2 cc. of antigen and 1 cc., then transferred from tube to tube containing 1 cc. of antigen.

3. A recently described method in which the three lowest dilutions were made by the first method and the remainder by the serum method. Of 150 tests set up by three persons, the second was found to be most accurate and least time consuming. L.D.B.

154. Der gelbe Galt und Versuche zur Heilung mit Entozon-Infusionen. (The Control of Bovine Mastitis by Use of Entozon.) R. HELM. Zschr. Infektkr. Haustiere. *50*: 4, 268–287.

This article is somewhat of a general discussion of the present methods of controlling this disease and the use of Entozon, an acridine dye. The animals used for study contained large numbers of streptococci and leukocytes in the milk and were considered as typical and satisfactory cows for study.

It appears that this treatment is of limited value. L.D.B.

155. Die Auswirkungen und Bekampfung der Geschlechtstrichomonaden in einer groben ostpreubischen Rinderherde. (Treatment and Control of the Trichomonads of the Genital Organs of a Herd of Cattle in East Prussia.) EULER. Zschr. Infektkr. Haustiere. *51*: 3, 203–215.

It is observed that trichomonad infections are becoming more numerous in Eastern Prussia.

The inflammatory reactions produced by these parasites are described. The use and limitations of the microscopic examination for detecting the disease are presented.

Methods of treatment for infected cows and bulls are given. Materials used in these treatments are Entozon 1–1000 to 1–1250. (Entozon is an acridine dye.) Lugol's solution and 1 per cent zinc sulphate, acetic acid and sodium carbonate, and a 1.5 per cent solution of valvanol. It is recommended that treatment be continued until microscopic examinations of the secretions are free of parasites. L.D.B.

156. Weitere Untersuchungen uber das Zellbild der Milch bei tuberkuloser Mastitis. (Mit 4 Abbidungen.) (Further Observations on the Cell Findings in Milk from Tuberculous Mastitis.) M. ZIEGLER. III Report. Zschr. Infektkr. Haustiere. *51*: (3), 176–185.

In the second report on this problem (Ziegler, M. and Schulze, W., Z. Fleisch-u. Milchhyg. 45, 5) concluded that the monocytes content of milk, which was described in their first report (Zeigler, M., and Bergmann, G., Z. Inf-krkh. Haustiere. 43, 211), was typical of tuberculous mastitis. The presence of the giant cell and epitheloid cell groups were of essential value as an aid to correct diagnosis. This present report is a further contribution in this field.

In the samples of milk examined, in which the giant cells or cell groups were demonstrated, they found tubercle bacilli in 36 per cent. In 218 other instances in which no tubercle bacilli could be demonstrated with the cells by microscopic methods, they were able to demonstrate them later by culture and animal inoculation.

In 226 udders examined after the cows were slaughtered, miliary tuberculosis was found 25 times, lobular infiltration 184 times, caseous mastitis 11 times, and 2 times the lesions were of mixed pathology.

They were able to demonstrate the giant cells and cell groups by microscopic study in about 50 per cent of the tuberculous animals studied.

The cell types described are the Langhan's giant cell, giant cells with nuclei irregularly distributed, epithelioidal-like cells which would probably become giant cells, and epithelioid cells in which there were but two or three nuclei. These cells were best demonstrated by low magnification.

It is believed that the cell picture may be used to advantage as an aid in the diagnosis of tuberculosis of the udder. L.D.B.

157. Beitrag zur Aetiologie der Rinderleukose in Ostpreussen. (A Report on the Etiology of Bovine Leukosis in East Prussia.) OTTO CZYMOCH. Zschr. Infektkr. Haustiere. *52:* (⅛) 187–220.

This condition has been reported under various names such as lymphomatosis, lymphoidomatosis, lympkosarcoma, leukemia and leukosis. The disease is widespread in East Prussia and appears to be on the increase. The cause is unknown but is considered by many to be of an infectious nature and spread through soil, feed, etc.

There appears to be no seasonal variation. The greatest incidence is in animals in four to six years of age, although it is observed in some animals of two years and over 13 years of age. Investigation showed that leukosis was much more common in animals pastured on loam soil, with sandy loam next. There was very little on moor or sandy soils. There is considerably more of the disease in the northern part of the area so that the region is of some significance. The distribution among wild animals is the same as among the cattle of the region. There was no definite relation between leukosis and parasitism, abortion disease, tuberculosis, or paratuberculosis. Forcing cows for milk production did not produce the disease.

The conclusion reached from this investigation is that bovine leukosis is a disease resulting from soil deficiencies. This depends on soil moisture. In those soils in which there is high ground water the fodder contains greater amounts of silicic acid and a deficient amount of other minerals. The long continued feeding of such grasses and hay will lead to inflammation of the intestinal mucosa and later to leukosis on those animals which possess an inherited predisposition to it. The conclusion is reached following an analysis of the soils, fodder, and blood of animals in various parts of the country. Also to the fact that it was not possible to transfer the disease by artificial means. L.D.B.

158. Stephanofilariosis in Niederländisch-Ostandien and ähnliche Infektionen in anderen Ländern. (Stephanofilariosis in Dutch East Indies and Similar Infections in other Lands.) F. C. KRANEVELD. Zschr. Infektkr. Haustiere. *53*: 4, 291–332. (42 illustrations.)

This is a discussion of the skin infestation of domesticated animals, chiefly cattle, in Sumatra, Java and other East India areas.

A classification of the parasites is given. A description of the parasite and the lesions produced by their infestation is presented.

Attempts to demonstrate an insect vector failed. No treatment was found effective. L.D.B.

159. Die Feststellung der Abortus-Banginfection (Bruzellose) des Rindes mit Hilfe serologischer Verfahren. (The Detection of Brucellosis in Cattle by Means of Serological Methods.) M. SEELEMANN AND A. PFEIFFER. Zschr. Infektkr. Haustiere. *53*: 3, 211–235

In this investigation two technics were used, one using the Sachweh extract and the second the Meinicke extract. (The method of preparing these so-called extracts is not published.) These methods were used with blood serum, whole milk, and milk serum.

The technic of the test is to set up the tubes in racks and to place 1 cc. of

the extract in one series of tubes and in a second series varying amounts of 5 per cent salt solution and antigen (0.02 to 0.3 cc. of antigen) to make a total volume of 10 cc. All the tubes are heated at 57° C. in water bath for a short period and the content of each pair of tubes together with 0.1 cc. of the proper dilution of the test serum added and the tubes centrifuged at 3000 r.p.m. for ten minutes. The flocculation sediment is then read. In a positive reaction the supernatant fluid is clear and the sediment collects in a button at the bottom of the tube. In negative tests the button is small and the supernatant fluid is cloudy. A similar technic is used for each type of extract except that for the Meinicke extract the tubes were centrifuged at 2000 r.p.m.

A complete flocculation occurred with a positive serum of 1–800 titre when the antigen was used in 0.1 cc. or greater amounts. When a serum of 1–6400 titre was used the same result was obtained with 0.03 cc. of antigen. The technic with whole milk and milk serum is the same as with blood serum.

It is concluded that the Meinicke extract is more satisfactory than that of Sachweh because the differences between positive and negative reactions are easier to read, although the Sachweh technic appeared to give a higher percentage of positive reactions when milk was used. In all three tests the uninfected animals gave completely negative reactions with all these methods. L.D.B.

160. Ist die Ratte an der Verbreitung des ansteckenden Verwerfens mitbeteiligt? (Is the Rat Concerned in the Distribution of Infectious Abortion?) A. SANDHOLM. Zschr. Infektkr. Haustiere. *53*: 3, 201–210.

In order to answer this question, five groups of rats were fed Brucella cultures. One group received the infectious organisms in milk for two consecutive days; a second in feces; a third received three highly virulent Brucella cultures suspended in milk; a fourth group were fed after-birth mixed with milk; a fifth group received similar material mixed with meal. The presence of an infection in the rat feces was determined by injecting them into guinea pigs. In group one, two rats developed agglutinins in 1–10 dilution in 9 and 12 days, and two others developed agglutinins in 10 and 11 days. In the guinea pigs inoculated with the feces of these animals, three developed agglutinins; one guinea pig became positive after being treated with rat feces obtained one day after it had received the contaminated milk, one guinea pig receiving feces from rats two days after feeding, and one from the rats seven days after feeding. None of the guinea pigs developed agglutinins after that date. Nothing developed in the second group of rats or in the guinea pigs which received their feces. In the group three rats, one guinea pig developed an agglutination titre following feeding of rat feces obtained the first day after the experiment started. Groups four and five were also negative.

The rat appears to be very resistant to this infection. In one instance the rat harbored the abortion bacillus in its intestine for at least two weeks so that it is a possible means of spreading the infection. L.D.B.

161. Die Rinderställe und die Verbreitung von Tuberkulose, seuchenhaftem Verkalben und gelbem Galt unter den Rinder eines sächsischen Dorfes. (The Influence of the Stable on the Dissemination of Tuberculosis, Abortion Disease and Mastitis.) H. HAUPT. Zschr. Infektkr. Haustiere. *53:* ½, 49–72.

This is something of a laboratory exercise in Veterinary Hygiene for certain students in Leipsig University. The introduction is by Haupt. The various sections are written by several others. The study was made in a small village near the city. The study included 25 stables and 280 animals, mostly cows (208). But 43.7% of these animals were entirely disease free; 6.7% were infected with abortion disease, 8.2% with mastitis, 28.3% with tuberculosis and the remainder with two or more diseases in combination.

It is concluded that the type of stall and the stable construction and management are of great importance in the dissemination of infectious disease among the animals occupying them. This is especially true in regard to the lighting arrangements. L.D.B.

162. Einfluss saurer und alkalischer Nahrung auf die Abortus-Bang-Infektion und auf Agglutininbildung. (Influence of the Use of Acid and Alkaline Feeds on Bang-infection and Development of Agglutinins.) ERICH LÜHRS. Zschr. Infektkr. Haustiere. *53*: ½, 43–48.

This investigation was undertaken because of the common practice of feeding silage during the winter season and green pasture during the summer. The former is considered to be an acid, the latter an alkaline diet. To determine the influence of feeding methods on abortion, records were taken on 1000 cows over two winters and one summer season. No influence was observed.

In a series of experiments using feeds with an average pH of 6.8, 7.3 and 7.5, there appeared to be no influence on the agglutinin titres of animals with abortion disease. L.D.B.

163. Ergebnisse der Untersuchungen über die praktische Verwendbarkeit einer Formolvakzine gegen das seuchenartige Verwenfen der Rinder. (Report on the Use of Formalized Vaccine in Control of Infectious Abortion.) FRITZ KRESS. Zschr. Infektkr. Haustiere. *52*: 4, 316–327.

The vaccine used was a fluid culture killed by formalin. Ten freshly isolated cultures were grown at 37° C. for two to four weeks. Two cc. of

formalin were added to each 1000 cc. and held at room temperature for 24 hours with frequent shakings before use. The first dose was 10 cc., after four weeks a second dose of 20 cc., and twelve weeks later a third dose of 20 cc. was used, although the time between doses might be reduced. There appeared to be little response to this treatment except that a slight rise in temperature which subsided within 24 hours was sometimes observed. There was also some swelling at the point of injection after the second and third treatments. There was little disturbance of the milk supply. The agglutination titre of the blood increased after each treatment but disappeared before the next treatment. About eight weeks after the last treatment it was slight in 1 to 50 but negative in 1 to 12.5 after 12 weeks. The milk did not exhibit agglutinins due to the treatment.

Among 1633 animals with early infection there were 39.8% reactors and 19.3 abortions. During the first period of pregnancy after the treatments, these abortions had been reduced to 0.9%. Among 762 animals with standing infection, there were 23.2% reactors and 15.9% abortions. After treatments the abortions had been reduced to 0.6%.

Emphasis is placed on the necessity of careful hygienic measures during the vaccination period. L.D.B.

164. Ueber eine Bartonella-Infektion beim Rinde. (A Bartonella Infection in the Ox.) O. NIESCHULZ. Zschr. Infektkr. Haustiere. *53*: 3, 175–179.

During an examination of the blood of a calf which had been inoculated with blood originally obtained in North Africa there was observed, besides anaplasma and iroplasma, a coccoid-like parasite situated within the red corpuscles. This was arranged 4 or 5 in a chain or in a mass of ten or more. The parasites may be arranged around the periphery of the cell. They were coccoid in form, 0.2 to 1.5 microns in diameter. These forms on the periphery are more darkly stained than those within the cell protoplasm. The parasite was very similar in appearance to *Bartonella canis* and may belong to the same genus, although it was finally identified with *Eperythrozoon wenyoni*. L.D.B.

165. Die Wichtigsten sudafrikanischen Giftpflanzen. (Important South African Poisonous Plants.) DOUW G. STEYN, Zschr. Infektr. Haustiere. *53*: 4, 233–349 (15 illustrations).

In the South African Union there are more than 200 poisonous plants. Several of these plants are described in this paper.

The animals commonly poisoned, the clinical symptoms and some pathological findings are given.

Plants causing photosensitization in animals with non-pigmented skins are listed with a description of symptoms which develop when these animals are exposed to sunlight. L.D.B.

FEEDS AND FEEDING

166. Digestible Nutrients and Metabolizable Energy in Certain Silages, Hays and Mixed Rations. F. W. CHRISTENSEN AND T. H. HOPPER, North Dakota Agr. Exper. Sta., J. Agr. Res., *57*: 477–512, October, 1938.

Digestion trials with six sheep (grade wethers) during 1923, with four steers during 1923–24, and with four steers in 1924–25 are reported. Roughages studied included sweet clover-oat straw silage (bud stage), sweet clover silage (bud stage), sweet clover silage (early bloom stage), sweet clover silage (full bloom stage), sunflower silage, corn silage, sweet clover hay and stems and alfalfa hay. Concentrates included linseed meal, barley and wild oats (removed from wheat for milling). Extensive data from balance trials with these feeds are given. Chemical compositions of the feeds are given.

The silages studied were generally higher in ash, crude protein, crude fiber, and ether extract, but lower in nitrogen-free extract than the crops when ensiled. On a dry-matter basis, the sweet clover silages and hays resembled alfalfa hay in percentage of digestibility, digestible nutrients, and metabolizable energy although the sweet clover silages averaged slightly higher in digestible crude protein and total digestible nutrients. Mixing 1 part of dry oat straw with 5.5 parts of green sweet clover reduced the digestible crude protein 56% and total digestible nutrients 37% in the silage, on a dry basis as compared to straight sweet clover silage from the same crop. The sunflower silages were less palatable than the sweet clover silages, which, in turn, were less palatable than the corn silage.

On a dry basis the average percentages of digestible protein and total digestible nutrients respectively for several of the feeds were: for the straight sweet clover silage, 16.2 and 56.8; for the corn silage, 5.3 and 69.7; for the sunflower silage, 4.8 and 47.7; for the linseed meal, 32.9 and 75.8: and for the wild oats, 9.6 and 61.6.

The metabolizable energy per pound of digestible organic matter in the different feeds and rations was fairly uniform. The average of 25 individual determinations on roughages was 1.737 therms per pound and 1.767 therms for 21 mixed rations. In 25 individual trials the average metabolizable energy per pound of total digestible nutrients was 1.670 therms, and in 21 trials of mixed rations, it was 1.689. L.M.T.

167. A Comparison of the Vitamin D Potency of the Stemmy and Leafy Portions of Alfalfa Hay. G. C. WALLIS, South Dakota Agr. Exper. Sta., J. Agr. Res., *57*: 393–396, Sept., 1938.

The leaves and stems of good quality, green-colored alfalfa hay, carefully separated, were found by the standard line-test technique, to contain approximately 10.45 and 1.72 International Units of vitamin D per gram,

respectively. The leaves represented 49.4% of the sample and the stems 50.6%. L.M.T.

168. The Carotene Content of Market Hays and Corn Silage. H. G. WISEMAN, E. A. KANE, L. A. SHINN AND C. A. CARY. Bureau of Dairy Industry, U.S.D.A., Washington, D. C. J. Agr. Res., *57*: 635–670. Nov., 1938.

A major portion of this paper describes the methods for the determination of carotene used in the laboratory of the Division of Nutrition and Physiology at Beltsville, Md., and presents data to show the accuracy of results obtained by these methods. The largest error was found to result from the inclusion of pigmented impurities other than xanthophyll in the final carotene extracts. However, it is shown that the methods yield a useful index of the vitamin A values of feeds for cattle.

The carotene contents of a number of roughages and concentrates were determined in addition to those for corn silages and various grades of market hays. Green, growing bluegrass and alfalfa averaged 567 and 332 mg. per kilogram, respectively, on a dry weight basis. On the basis of weight as fed the following average values per kilogram were found: artificially dried alfalfa leaf meal, 151 mg.; corn silage, 14 mg.; alfalfa hay, U. S. No. 1 in color, 43 mg.; alfalfa hay, U. S. No. 2 in color, 15 mg.; alfalfa hay, U. S. No. 3 in color, 3 mg.; timothy hay, U. S. No. 1 in color, 21 mg.; timothy hay, U. S. No. 2 in color, 9 mg.; timothy hay, U. S. No. 3 in color, 5 mg.; yellow garden carrots, 91 mg.; dry corn stover, 4 mg.; and clover hay, U. S. No. 1, 23 mg. Considerable variation occurred with individual samples, averages for which are shown here. L.M.T.

169. The Level of Inorganic Phosphorus in the Blood of Dairy Cattle. S. R. JOHNSON, Inst. of Nutrition, Pa. State College, J. of Nutrit. *17*: 15–21, 1939.

The purposes of this investigation were to establish standard normal values for the inorganic phosphorus of whole blood of dairy cattle, and to study the variations resulting from differences in conditions as to breed, age, milk production and reproductive status. Inorganic phosphorus was determined in the whole blood of more than 200 normal cattle.

The values for calves from one to seven months averaged 6.42 mgm. per cent. There was a gradual decrease with age. Mature cows averaged 4.29 mgm. per cent. Breed of cow, the stage of lactation or the kind of roughage consumed did not affect the inorganic phosphorus content of whole blood. C.F.H.

170. Evaluation of the Vitamin A Potency of Feeds. G. S. FRAPS, Texas A. & M. College, College Station, Texas. J. Ind. & Eng. Chem., Analyt. Ed. *10*: 9, 525–27, 1938.

The author discusses the importance of making vitamin A determinations

in feed and reviews the methods available for the estimation of carotene therein. B.H.W.

171. **Additional Nutritional Factors Required by the Rat.** J. J. Oleson, H. R. Bird, C. A. Elvehjem, and E. B. Hart, J. Biol. Chem. *127*: 23, 1939.

Four different deficiency symptoms have been produced in rats on synthetic diets low in the vitamin B complex but adequate in thiamine, riboflavin, choline and nicotinic acid. Crystalline B_6 was very active in preventing acrodynia, but inactive in the preventing of paralysis, hemorrhage disease, or spectacled eye condition. The relation of these deficiencies to known members of the B complex is discussed. The importance of dietary fat and carbohydrate in relation to growth was discussed. K.G.W.

172. **Carotene, Isolation, and Detection of Carotene, and the Carotenes of Carrot Roots and Butter.** Harold H. Strain, Carnegie Institution of Washington, Div. of Plant Biology, Stanford Univ., Palo Alto, Calif. J. Biol. Chem. *127*: 191, 1939.

Since the carotenes of butter vary with those ingested by the animals, investigations of the butter pigments must be supplemented by investigation of the carotene of the rations. Occurrence of only B carotene in butter after cows had received principally this pigment in the rations indicate that carotenes are not interconvertible in the cow. The proportions of the carotene isomers isolated from butter after cows had been fed carrots were similar to the proportions of the carotenes in the ration. The rapidity with which the carotenes of carrot roots are transferred to butter suggests that carrots may be used as a source of vitamin A in milk and butter in northern and arid regions where green fodder is not available.

Carrots contain considerable quantities of a carotene pigment the spectral absorption properties of which are similar to those of the flavoxanthins. This pigment does not occur in appreciable amounts in the leaves. K.G.W.

173. **The Effect of Different Methods of Drying on the Biological Value and the Digestibility of the Proteins and on the Carotene Content of Grass.** Stephan Bartlett, K. M. Henry, S. K. Kon, L. W. Osborne, S. Y. Thompson and Joseph Tinsley, Nat. Inst. Res. in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. Biochem. J. *32*: 2024, 1938.

One of the outstanding features claimed for modern methods of artificial grass drying is that they involve but little destruction of the nutritive properties of the grass. The high temperatures attained during the process, however, raise the question of heat injury to the proteins. It is well known that heating lowers the biological value of the nitrogen of various foodstuffs.

Nitrogen balance experiments with rats were carried out using grass dried by artificial means and also made into hay the normal way and the effect of the various procedures on the carotene content and on the biological value of the proteins of grass were measured.

The biological value and true digestibility of the proteins of grass artificially dried at the normal running temperature (300° F.) in a rotating drum type drier were compared on rats with those of grass dried at low temperature (170° F) and those of sun cured hay. All three samples were made from one bulk of fresh grass.

The following figures were found for biological value and true digestibility; artificially dried grass, high temperature, 67 and 66; artificially dried grass, low temperature, 62 and 65; hay, 52 and 64.

The differences in biological values of the proteins between artificially dried samples and hay were statistically highly significant. Other differences were not.

The hay cured under natural conditions contained only about one-quarter of the carotene originally present in the fresh grass. Artificial drying at normal temperature preserved nearly 90% of the carotene. K.G.W.

174. Relation of Roughage to the Fat Content of Milk. E. B. POWELL, Ralston Purina Company, St. Louis, Mo. Cert. Milk, *13*: 152, 9, 1938.

It was found that some long range fat variations are related to the physical characteristics of the ration. The physical condition of the roughage when it is consumed influences the fat content of the milk. The author is of the opinion that there is a definite correlation between the activities of the rumen and the composition of the milk which a cow produces. W.S.M.

175. Urea as a Protein Substitute in the Diet of Young Cattle. S. BARTLETT AND A. G. COTTON, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res. *9*: 263–272. 1938.

Twenty-one dairy heifers aged 7 to 17 months were used in a 42 day feeding experiment in which part of the animals received a normal-protein ration, part a low-protein ration, part the low-protein ration supplemented with urea in sufficient quantity (0.127 pounds per animal per day) to supply the same total nitrogen as the normal-protein nitrogen, and part the low-protein ration in which a small quantity of starchy food was replaced by ground-nut protein, making the total nitrogen content equal to the normal-protein ration.

Animals on the urea supplemented ration had an average gain in liveweight of 0.24 pounds per day more than those on the low-protein diet. This increase was determined to be statistically significant; therefore, the authors

conclude that the animals appear to have utilized the urea in their metabolism. The animals receiving the same quantity of additional nitrogen in the form of protein showed slightly better live-weight gains than those receiving urea. S.T.C.

176. What Is the Truth about Grass Silage? J. W. BARTLETT, N. J. Agr. Exper. Sta., New Brunswick, N. J. Cert. Milk, *13*: 149, 9, 1938.

It is suggested that the value of grass silage be expressed as "nutrients per acre" and not just tons per acre. W.S.M.

177. "Canning" of Legumes and Grasses. L. J. TOMPKINS, Sheffield Farms Co., New York City. Cert. Milk, *13*: 152, 7, 1938.

A discussion of the following principal methods of curing forage: (1) natural or sun curing; (2) artificial dehydration; (3) silage green crops; (a) by natural fermentation, (b) by addition of acids, (c) by addition of molasses. W.S.M.

FOOD VALUE OF DAIRY PRODUCTS

178. The Biological Value of Carotene in Various Fats. E. J. LEASE, J. G. LEASE, H. STEENBOCK, AND C. A. BAUMANN, Dept. of Biochemistry, Univ. of Wis. J. of Nutrit. *17:* 91–102, 1939.

The biological response of rats to carotene administered in various oils, using the growth, cure of ophthalmia and the storage of vitamin A in the liver as biological criteria was studied. When excessive amounts of carotene or vitamin A were fed in lard, soybean oil, cottonseed oil, devitamized butterfat and a hydrogenated vegetable fat, there was no marked difference in storage. One microgram of carotene in cottonseed oil, soybean oil, decolorized butterfat, coconut oil or crude peanut oil gave approximately equal growth responses. Inferior growth was obtained on triolein, linseed oil and "refined" peanut oil. The rate of cure of ophthalmia was essentially the same for all oils. C.F.H.

179. Planning the Best Diet for the Family. E. V. MCCOLLUM. Milk Plant Monthly *27*: 12, 23, 1938.

The author points out that good nutrition is easily attainable in this country. A wider knowledge of the way to plan the diet, and an appreciation of the importance of good food in relation to health is essential.

A diet consisting of white bread, refined cereals, excessive sugar and sweet foods together with potatoes and lean meat will not be adequate for the maintenance of health. A proper amount of protective foods when included in the daily menus will supply everything that is needed for the promotion of health.

The daily food must supply the proper kinds and amount of proteins, minerals, and vitamins.

The value, necessity and source of vitamins A, B, C, and D are discussed.

Each member of the family should have daily a quart of milk or its equivalent, two salads, two servings of cooked leafy vegetables, one serving of meat, fish or poultry, at least four eggs a week, and some fresh fruit or fruit juice. G.M.T.

180. Assays of Riboflavin and the Filtrate Factor in Certain Milk Products. T. H. JUKES AND G. A. RICHARDSON, California Agr. Exp. Sta., Davis, Calif. J. Agr. Res., *57*: 603–610, Oct. 1938.

In November, 1935, and again in May, 1936, a large amount of milk produced in the same territory, but not necessarily from the same herds, was divided and manufactured into the various dried products enumerated below. Results of chemical analyses of these products are given. Assays of the riboflavin and filtrate factors of each of the products were made, using chicks 6 to 8 days old as the experimental animals. The assays did not show any differences between the November and the May samples. The descending order of potency in filtrate factor for the November samples was: rennet whey, sulphuric acid casein whey, lactic acid casein whey, hydrochloric acid casein whey, buttermilk, skimmilk and whole milk. The order for the May samples was the same except that buttermilk was slightly superior to hydrochloric acid casein whey. In round numbers the dried wheys of various types furnished 3 to 6 units of filtrate factor per gram; dried buttermilk, 3 to 4; dried units of filtrate factor per gram; dried buttermilk, 3 to 4; dried skimmilk, 2 to 3, and dried whole milk, 1 to 2. The dried products furnished two to five times as much riboflavin as filtrate factor. L.M.T.

181. The Relation of Color and Carotene Content of Roughage in the Dairy Ration to the Color, Carotene Content, and Vitamin A Activity of Butter Fat. R. E. HODGSON, J. C. KNOTT, AND H. K. MURER, Washington Agr. Exp. Sta., and R. R. GRAVES, Bureau of Dairy Industry. J. Agr. Res., *57*: 513–529, Oct., 1938.

Three groups of 10 Holstein-Friesian cows each were winter-fed respectively on field-cured hay from perennial grass and clover mixtures, on silage made from the grass and clover mixtures and silage made from oats and peas, and on a combination of the hay and silage. All the cows were maintained on pasture alone during the summer. The color (green) and the carotene content of the roughages were determined and compared with the color (yellow), carotene content and vitamin A activity of the butterfat produced. Vitamin A determinations were made by a modification of the Sherman method. Results show that the hay was the poorest source of vitamin A in the butterfat among these roughages and grazed grass the best,

while the silages were nearly as good sources as the grass. There was neither a consistent relationship between the carotene content of the feed and that of the butterfat produced from it nor between the carotene content of the butterfat and its color. However, average values showed an increase in the color of the butterfat as the carotene content increased. The carotene in the butterfat accounted for only 11.2 to 13.7% of its vitamin A activity according to calculations by the authors. L.M.T.

182. The Influence of Various Fodder Supplements on the Production and Nutritive Value of Winter Milk. S. BARTLETT, A. G. COTTON, K. M. HENRY, AND S. K. KON, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res., *9*: 273–309, 1938.

An experiment is described in which comparisons were made between the effect of (a) mangels (control feeding), (b) artificially dried grass, (c) sprouted maize, and (d) kale used as dietary supplements on the production and nutritive properties of milk of dairy cows. No significant differences were found between any of the foods in their effect on yield of milk, or its content of fat or solids-not-fat. The vitamin A and carotene contents of the rendered butterfat were measured spectrophotometrically and with the Lovibond tintometer. The feeding of kale and artificially dried grass markedly increased the color and vitamin A content of the milk, but sprouted maize had no more effect in this respect than mangels.

The vitamin B complex level of the milk was not measurably influenced by the feeding of any of the fodders, but the vitamin B_2 (complex) level and vitamin C level was lowest in the milk from the cows fed sprouted maize. The growth-promoting properties of the milk produced on mangels, artificially dried grass and sprouted maize were compared. The milks were fed, supplemented either with minerals alone or with minerals plus sugar as a source of energy. In one series of experiments the milks were given ad libitum, in another the intake was "equalized." No differences were found and the authors concluded that under English conditions this technique does not disclose the presence in milk of a new essential factor. S.T.C.

183. The Vitamin A Value of Colostrum and Milk of Four Cows Determined by the Single-feeding Method. M. M. KRAMER, MARGARET D. BLAIR, AND W. H. RIDDELL, Kansas Agr. Exp. Sta. J. Agr. Res., *56*: 227–232, Feb., 1938.

Total vitamin A values, found by the single-feeding biological method of Sherman, were determined for the colostrum milk of two cows on the regular dairy herd ration and for two cows on the same ration supplemented with rye pasture. The milks of the second day of lactation were highest in vitamin A value, 25 and 28 International Units per gram for the cows on rye

pasture and 16 and 20 units for the others. The vitamin A value was considerably reduced by the fourth day and from the seventh day until the end of the month of testing the value remained fairly constant for the four cows at approximately 2 International Units per gram. Comparisons of the total production of vitamin A per day proved highest on the first or second day of lactation. One of the cows receiving no supplement maintained the highest level of vitamin A output during the latter part of the month. In general the total daily production of vitamin A was quite constant from the end of the first week until the end of the month. L.M.T.

184. Vitamin G (Riboflavin) Content of Colostrum and Milk of Cows Determined Biologically. M. M. KRAMER, BERNICE L. JUNNERTH, AND W. H. RIDDELL, Kansas Agric. Exper. Sta. J. Agr. Res., *56*: 233–237, 1938.

Twenty-four hour composite samples of colostrum milk from two Jersey and two Holstein cows on the first, fifth and thirtieth days of lactation, together with a sample of mixed herd milk, were tested for vitamin G by biological assay. Flavin reference curves were used to estimate the vitamin G content per gram. First day colostrum was richest in vitamin G and was estimated to contain 4.2 to 6.7 micrograms per gram. A gram of colostrum, 2 grams of fifth-day milk, and 3 grams of thirtieth-day milk gave approximately the same gains in weight for the groups of rats fed. Herd milk was estimated to contain about 2 micrograms per gram. The largest total daily outputs occurred on the fifth day, but these totals approached the yields for the thirtieth day. L.M.T.

185. Improving the Curd Size of Certified Milk. I. J. WOLMAN, Univ. of Pennsylvania School of Medicine, Philadelphia, Pa. Cert. Milk, *13*: 150, 5, 1938.

A general discussion of methods for producing artificial soft curd milk. The nutritive benefit of adequately homogenized milk, whether pressure treated or sonized, seems to rest in the small curds that form in the stomach. Other advantages of homogenization are: improved flavor, uniform distribution of fat, and protection against theft of cream content.

The author suggests that certified milk producers check the curd forming qualities of their product at regular intervals and report these findings to the Medical Milk Commissions. W.S.M.

186. New Deal in Nutrition Necessary for Recovery. J. A. TOBEY, American Institute of Baking, New York City. Cert. Milk, *13*: 150, 7, 1938.

An adequate diet is defined. The present American diet is discussed and an improved national diet is suggested. W.S.M.

187. Vitamin C Content of Certified and Other Grades of Market Milk. C. C. DAVIS, The Medical Milk Commission of the New Orleans Pure Milk Society, New Orleans, La. Cert. Milk, *13*: 152, 5, 1938.

Using the rapid titration method as described by Sharp for determining vitamin C content of milk, data from a study of certified and other grades of market milk are presented.

The average value found in certified raw milk was about one-fourth higher than in Grade A raw, nearly twice as high as in special Grade A pasteurized, and five and one-half times as high as in regular Grade A pasteurized milk.

Bright daylight and especially direct sunlight was found to be very destructive to vitamin C in milk, even when exposed but a short time.

Fresh milk stored in a refrigerator lost about 45 per cent of this factor in 72 hours. W.S.M.

ICE CREAM

188. Consumer Preference for Ice Cream. H. E. ROBERTS AND R. B. STOLTZ, Ohio State Univ., Columbus, Ohio. Ice Cream Rev., *22*: 6, 40, 1939.

The results of a consumer quantity preference experiment in which people were allowed to eat all the ice cream they wanted are given. The results were checked against the questionnaire and sample method. In general it was found, the two methods gave similar results; however, there were some differences. For example, by the questionnaire and sample method, consumers expressed a preference for a high serum solids ice cream, but when allowed to eat all the ice cream they wanted, as was done under the quantity test, consumers ate more of the lower serum solids ice cream. Other variants so studied were color, per cent of sugar, egg yolk, per cent butterfat, type of vanilla and kind of sugar. Questionnaire and sample results also are given for consumer preference of mixes containing noticeable "off" flavors. J.H.E.

189. Low-Lactose Milk Solids in Ice Cream. W. J. CORBETT, Univ. of Ill., Urbana, Ill. Ice Cream Rev. *22*: 6, 34, 1939.

A procedure is given for the preparation of low-lactose milk solids taking advantage of the wheying off obtained when a derivative of the Carob bean is added to milk. The use of 3.5 to 4.5 per cent of the low-lactose solids in addition to 8.5 to 9.5 per cent of regular serum solids produced an improvement in the body of ice cream frozen in a batch freezer. Replacing one-fourth of the sucrose in low-lactose mixes with dextrose is recommended to prevent the ice cream from having a dry body. J.H.E.

190. Keeping Store Operations Under Control. VERNE RIGHTER, Barber Bros. Co., Chicago, Ill., Ice Cream Rev., *22*: 6, 37, 1939.

In the operation of retail ice cream stores, careful observation of the money handling, frequent checking of the register, taking inventory, and figuring dippage, alternating the employees, and a knowledge of the environment of each clerk, are recommended to prevent losses. J.H.E.

191. Colors and Flavors. D. LEVOWITZ, New Jersey Dairy Laboratories. Ice Cream Field, *32*: 10, 95, 96, 98, 1938.

Directions are given to aid in the preparation and care of colors and flavors for use in ice cream.

The following points are emphasized:

1. A perfect mix may be ruined by materials added at the freezer.
2. Colors and flavors, fruits and nuts should be selected carefully and should be stored and handled in a sanitary manner.
3. Unless controlled, materials added at the freezer can nullify the protective steps in processing the mix. W.C.C.

192. Refrigeration Problems. A. M. SCHMITZ. Servel Inc. Ice Cream Field, *32*: 10, 94, 99, 1938.

Brief instructions are given as to seasonal adjustments in ice cream cabinets, also as to means of overcoming the difficulty of Freon compressors on ice cream cabinets pumping oil out of the crankcases, thereby resulting in damage to the bearings and moving parts. W.C.C.

193. Egg Yolk Helps Sales. J. R. TURNER, Armor & Co. Ice Cream Field, *32*: 10, 29, & 30, 1938.

The author refers to a survey by Tracy and Smith of the University of Illinois in which it is claimed that the majority of the ice cream eaters prefer ice cream with high serum solids and medium fat contents. They prefer ice cream containing a stabilizer and also ice cream that is medium in color.

The author makes the following claims for egg yolk as an ingredient in ice cream.

1. The emulsifying properties of egg yolk produce small uniform air cells.
2. Egg yolk makes possible an increase in serum solids without causing sandiness. (Note: To the knowledge of the reviewer this point has not been established.)
3. Egg yolk gives ice cream a natural rich appetizing color.
4. Egg yolk permits a reduction in butterfat and improves the flavor without sacrificing sales appeal.
5. Use of egg yolk in ice cream permits a reduction in the amount of stabilizers to a satisfactory basis without a loss in body and texture.

The author claims that many people are wrong in assuming that the principal advantage of egg yolk in ice cream is improved whipping ability of the mix. He claims further that egg yolk powder is more convenient to use than other forms of egg products. W.C.C.

194. Refrigeration Problems. A. M. SCHMITZ, Servel Inc. Ice Cream Field, *32:* (12) 14, 16, 17, 18, 1938.

Eight standards are given for rating condensing units which were adopted in 1937 by the American Society of Refrigeration Engineers, Refrigeration Division of the National Electric Manufacturers Association and the Refrigerating Machinery Association. The author points out that unless the suction vapor is superheated to the same temperature used by the manufacturer in rating the unit, actual capacity may deviate from the rated capacity by as much as 15 per cent. Examples are used to illustrate this point.

The functions of a compressor and evaporator are briefly described and the advantages of using a heat interchanger are also indicated. The heat interchanger is a device consisting of two copper tubes and necessary fittings so arranged that the suction vapor from the evaporator flows through the inside tube and the warm liquid refrigerant from the receiving flows in the opposite direction in the space between the two tubes. This serves to transfer some of the heat from the liquid refrigerant to the cold suction vapor. This results in a silght increase in the condensing capacity as well as a saving in the amount of liquid refrigerant which is evaporated to cool the liquid to the evaporator temperature. In a specific example the author shows an 11 per cent saving as a result of the use of the interchanger. W.C.C.

MILK

195. Manual for Milk and Cream Testers in Maryland. C. W. ENGLAND, Maryland Agricultural Experiment Station, Bulletin 423, December, 1938.

This bulletin is a revision of Parts I and III only, of the Maryland Agricultural Experiment Station Bulletin 401. It describes practical and approved methods of testing fresh milk, preserved composite samples of milk and cream by the Babcock Method, and is designed to aid those persons desiring to pass examinations for Babcock tester's licenses on milk and cream. It also contains specifications for Babcock glassware and the Dairy Inspection Law of Maryland. C.W.E.

196. Die Bekampfung der Abortus-Bang-Infektion, der Tuberkulose und des Gelben Galtes im Plane der Mabnahmen der Rohmilchversorgung. (Control of Abortion Disease, Tuberculosis, and Mastitis in Order to Protect the Raw Milk Supply.) DR. PLATE. Zschr. Infektkr. Haustiere. *50:* 4, 287–303.

A general discussion of the milk supply of the city of Bremen. An improvement in health condition, and decrease in infant mortality, followed the elimination of the above disease from the dairy herds of that city. There is a general discussion on methods of control of those infections, and their importance as diseases in man. L.D.B.

197. The "Lactotest" of Koschuscharoff. A More Simple Method of Determining the Addition of Cow's Milk to Woman's Milk. MARIE-ELISE KAYSER. Regional Clinic for Women at Erfurt. Le Lait *18*: 179, 930–934, 1938.

By means of a specially prepared serum called "lactotest," it was possible to detect the addition of relatively small amounts of cow's milk to human mother's milk. The mother's milk to be tested is first centrifuged to remove fat. A drop of the defatted milk is then mixed with 4 times its weight of the serum on a microscope slide. With pure mother's milk, the drop appears uniformly turbid under the microscope with a whitish cloud at the center of the drop. When cow's milk is mixed with mother's milk, a positive reaction is given after ½ minute. The liquid which was turbid at the beginning became clear; especially the peripheral liquid, with a definite coagulation. This method was considered superior to several colorimetric methods that were tested. It did not require a control, slight adulterations of mother's milk with cow's milk could be detected, expensive reagents were not required, and the reaction was rapid. A.H.J.

198. The Hygiene of Milk in Canada. E. U. ALLARD, Chief Inspector of Milk, Quebec, Canada. Le Lait *18*: 179, 919–930, 1938.

The advantages of pasteurized milk are emphasized. Raw milk is usually much more variable in composition than pasteurized milk. Of more than 3000 samples of raw milk tested, about 26 per cent were below the legal standard of 3.25 per cent fat, while for pasteurized milk only 3 per cent were below the legal fat requirement. The reason for the variation is due to the large amounts of mixed milk that go into pasteurized lots of milk while raw milks are in many cases only the mixed milks from single farms. A.H.J.

199. Treatment and Transformation of Milk, Improvement in Quality. W. CLAUSS, Agronomic Engineer, Berlin, Germany. Le Lait, *18*: 179, 982–999, November, 1938.

Methods of handling milk on the farm and processing it in the dairy so that better market milk, butter, cheese, and other dairy products can be produced from it are discussed. Comparative practices in various European countries and in the United States were outlined. A.H.J.

200. Shipping Milk in Track-Rail Steel-Lined Tanks. ANONYMOUS. Ice and Refrig. *94*: 1, 71, 1938.

Some railroads, *e.g.*, the N. Y., Ontario and Western have transported

milk in special 2,500 gallon steel lined tanks which are transferred from truck to flat cars. Four or five tanks are loaded on a single flat car and are again transferred to trucks on arrival in the city. The tanks are readily transferred and may be used as storage tanks in the plant. The inner stainless steel shell is covered with 2½ inches cork insulation and a steel outer shell. L.C.T.

201. Practical Value of the Resazurin Test. ANDREW MOLDAVEN. Milk Plant Monthly, *27*: 10, 58, 1938.

Tests were carried out with the purpose of finding out if the resazurin test was consistent from a practical standpoint, to grade milk supplies of "unknown" origin according to their sanitary and keeping qualities.

The results showed that advanced cases of mastitis, "wet" milking, milk handled in poorly sterilized utensils or cans, faulty cooling and other unsanitary conditions can be detected by the resazurin test.

The resazurin test also can be used to differentiate certain off-flavors in milk such as feed, from those due to bacterial action, by showing good keeping qualities of the former.

The simplicity, rapidity, and dependability of the test make it a valuable one in the improvement in the quality of raw milk supplies. G.M.T.

202. The Chloride Content of Milk. W. L. DAVIES, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res., *9*: 327–335. 1938.

Chloride analyses were made on 7802 samples of milk principally from the Shorthorn and Guernsey cows in the Institute herd, though results from a herd of Friesian and a herd of Ayrshire as well as other samples are included. The modal value for Guernsey and Shorthorn milk was found to be 71 to 80 mg. per cent and for the Friesian milk 111 to 120 mg. per cent. Morning milk was found to have a higher chloride content than evening milk as the mode for morning milk was 81 to 90 and for evening milk 71 to 80 mg. per cent. The data indicate that milk from successive lactations of a cow increases in chloride content. Periods of drought in summer caused an appreciable increase in the chloride content of the milk. The effect of changing from winter feeding to spring grass, however, was to lower the chloride content. With advancing lactation the chloride content increased slowly during the period when milk yield is relatively high. Usually after the seventh month, however, the chloride content of the milk rose rapidly.

The electrical conductivity at 18° C. of over 100 samples of milk of chloride content ranging from 61 to 235 mg. per cent was determined by the bridge method. The electrical conductivity of milk was found to increase with chloride content, however; in samples high in chloride the electrical conductivity increased at a greater rate than the chloride content. S.T.C.

203. The Methylene Blue Reduction Test and the Keeping Quality of Milk. C. H. CHALMERS, The University, Leeds, England. J. Dairy Res., *9*: 351–355. 1938.

The keeping quality of 200 samples of milk was determined as the time required for the milk to develop an unpleasant flavor on incubation at 15° C. (59° F.). The Methylene Blue Reduction test was made on different portions of the same milk samples after incubation for one hour at room temperature and after incubation for 7, 16, and 23 hours at 15° C. (59° F.).

The correlation coefficients for the reduction time and the keeping quality for the different age periods at which the reduction test was started were as follows:

Age of the milk when tested	Correlation between the reduction time and the keeping quality
hr.	
23	0.954 ± 0.0043
*16	0.893 ± 0.015
7	0.660 ± 0.055
1	0.442 ± 0.059

* Only 75 samples tested.

Thus the author concludes that by incubating the milk for a period of 15 to 24 hours at 15° C. (59° F.) a much better correlation can be obtained between the reduction time (at 37° C. (98.6° F.)) and the keeping quality, than if the milk is examined within a few hours of its production. S.T.C.

204. Physical State of Fat and Losses in Centrifuging. HAROLD E. ROSS, Milk Plant Monthly *27*: 12, 30, 1938.

Eighty-pound samples of milk were divided into two lots of 40 pounds each. One lot was cooled to 50° F. and held for 30 minutes, while the other lot was heated to 115° F. for 30 minutes. Both lots of milk were then adjusted to a temperature of 85° F. and run through the same separator.

Samples of cream and skimmilk were collected from each lot and tested for fat.

Thirty of 31 samples showed a greater loss of fat in the skimmilk from the samples warmed from 50° F. to 85° F. than the skimmilk from the samples cooled from 115° F. to 85° F.

The average difference of fat loss between the two lots of skimmilk was 0.028 per cent by the Mojonnier method. So far as could be determined the controlling factor was the physical condition of the fat. G.M.T.

MISCELLANEOUS

205. Carbon Dioxide in Its New Field of Usefulness. J. C. GOOSMANN, Dr. Eng., Carbonic Engineering, New York, N. Y. Ice and

Refrig. *91*: 6, 462; *92*: 2, 130; *92*: 3, 208; *92*: 4, 285; *92*: 6, 434; *93*: 4, 279; *93*: 5, 359.

This is a continuation of abstract previously reported. (See abstract Number 154, J. OF DAIRY SC., Vol. 20, No. 5.) Methods for the purification and dehydration of carbon dioxide gas, which represent two important steps in the manufacture of dry ice, are discussed in considerable detail. Equipment for solidification of liquid carbon dioxide is diagrammed and illustrated. A description, together with illustrations of the Mathieson Alkali Manufacturing plants, is included. L.C.T.

206. Survey of Cold Storage Locker Plants. ANONYMOUS. Ice and Refrig. *93*: 4, 271, 1937.

Operating data for 48 locker plants is included. The number of lockers vary from 100 to 625. Monthly rentals vary from seventy-five cents to $1.50, and yearly rentals from $7.50 to $10.00. Practically every plant has a butcher in charge. Locker plants are operated largely in connection with other enterprises, *e.g.*, creamery, ice and cold storage, service stations, etc. The population of towns where located varies from 200 to 52,000. Distances served range up to 20 miles. Lockers are rented mostly by farmers, although up to 40% of the space in some instances is rented to those living in the city. A few who report the cost of electricity and water estimate it at $1.50 to $2.20 per locker per year. A list of precautions to be observed in the successful operation of a locker system include such items as a butcher in attendance, sharp freezing of meat before it is placed in the locker, no seasoning be used in the meat, rapid handling, build a well insulated plant, not too large, avoid monthly renters, encourage a three month turnover of meat in order to avoid refrigerator burn, and investigate thoroughly before embarking on the enterprise. L.C.T.

207. Cold Storage Locker Plants. A. L. BLATTI, Chief Eng. Railways Ice Co., Chicago, Ill. Ice and Refrig. *92*: 5, 371, 1937.

The cold storage locker industry had its origin on the Pacific coast about six years ago. Now there are more than 6,000 in existence. The layout of a locker plant should consist of a processing room to be held at 34° F., a sharp freezing room at −10° F. and a locker room maintained at +10° F. Floor plans for locker plants are included. L.C.T.

208. Automatic Controls—Results Obtained from Their Modern Application. W. E. ZIEBER, York Ice Machinery Corp., York, Pa. Ice and Refrig. *94*: 2, 114; *94*: 3, 207, 1938.

The author discusses briefly the differences between hand, semi-automatic, and automatic control of refrigeration systems, and points out (a) that direct

acting controls are those in which a fluid is used in a thermal element, which through a piece of tubing causes a pressure change directly on the valve or motor to be moved, either by hydrostatic pressure or gas pressure; and (b) that an air operated system consists of small thermally operated valves which pass air through them from a central system. The air pressure in turn functions upon the various pieces of equipment; and (c) finally a control system in which the thermal element makes or breaks an electric circuit, which moves the mechanism to bring the various parts into play.

The discussion is illustrated with a number of charts and diagrams showing detailed locations of controls as they might be used in a dairy plant.

The writer points out the changes that have taken place in controls in recent years. L.C.T.

209. Plant Problems, Their Importance and Solution. FRED J. HOPKINS. Ice and Refrig., *92:* 3, 219, 1937.

A list of points which should be observed in order to reduce operating costs in the manufacture of ice are included as follows:

1. The existence of non-condensable gases in the ammonia system.
2. Insufficient charge of ammonia.
3. Ammonia leaks.
4. Improper degree of flooding of evaporator systems.
5. Low brine level in freezing tanks.
6. Low specific gravity of brine.
7. Improper condition of brine (either acid or too alkaline).
8. Improper submergence of cans in brine.
9. Improper water treatment (or no water treating system).
10. Lack of properly calibrated instruments (gauges and thermometers).
11. Uninstructed operating personnel.
12. Operation of obsolete equipment.
13. Unbalanced plant conditions.

L.C.T.

210. Fiber-Like Merino Wool Made from Casein. ANONYMOUS. Milk Plant Monthly, *27:* 9, 46, 1938.

A synthetic fiber having the appearance of wool can be manufactured from casein by a process similar to that used in making rayon from cellulose. This fiber has a chemical composition almost identical with wool; is faintly yellow in color and closely resembles the best grade of washed and carded merino wool. It has the characteristic kink of wool, but lacks the scaly nature of wool fiber. Soft and harsh fibers may be made by varying the manufacturing process. Both the fineness and length of the fiber is regulated in the process.

It is believed that it can be manufactured to be sold at a price on a par

with rayon. The agents used for casein wool manufacture are cheaper and may be used longer than those used in rayon manufacture.

Casein is made from skimmilk. Whether or not the skimmilk will be more valuable for the feeding of calves and hogs, or manufactured into fiber remains to be determined. G.M.T.

211. Sterilization By Radiation. O. F. GARRETT AND K. B. ARNOLD. Milk Plant Monthly, *27*: 8, 39, 1938.

The possibilities of sterilizing with ultra violet light are pointed out in this article.

Data are presented which show that great decreases in bacterial counts are encountered on surfaces exposed to ultra violet light. The effectiveness of sterilization depends upon the types of bacteria present, time of exposure and distance from the source of the rays to the object being treated.

It seems possible that the method may be applied to equipment and operations in the dairy plant. G.M.T.

212. Applications of Nickel and Its Alloys. F. RENAUD. Le Lait *18*: 179, 1004–08, 1938.

The fabrication of various types of dairy equipment from nickel, inconel, and particularly from stainless steel (18% chromium and 8% nickel) is discussed. A.H.J.

PHYSIOLOGY

213. Observation on Specific Nutritional Factors in Lactation. S. J. FOLLEY, W. IKIN, S. K. KON, AND H. M. S. WILSON, Nat. Inst. for Res. in Dairying, Univ. of Reading, Reading, England. J. Biochem., *32:* 1988, 1938.

The results of this investigation indicate that rats can lactate normally on simplified diets. No evidence was obtained of the existence of specific dietary factors stimulating lactation qualitatively different from the dietary principles normally received by the non-lactating rat.

A simplified diet of ground rice, casein, butter, salts and yeast was found to produce satisfactory lactation in rats. Additions of fresh liver, liver extract or yeast were without effect on lactational performance. Fairly satisfactory lactational performance was obtained on a similar diet in which casein was replaced by fish protein. Stock colony rats which failed to rear their first litters were found to be more likely to lose their second litters than rats which successfully raised their first litters. Subcutaneous injection of tablets of pig anterior pituitary into such rats just after the second parturition had no effect on their ability to rear their young. K.G.W

214. The Diffusion of Trimethylamine Oxide from the Udder. W. L. DAVIES, National Institute for Research in Dairying, Univ. of Reading, Shinfield Nr. Reading, England. J. Dairy Res., *9*: 323–326. 1938.

A neutral solution of pure trimethylamine oxide was injected into two quarters of an udder. The quarters increased in turgidity up to 2.5 hours and then relaxed. The oxide diffused rapidly out of the quarters during the third and fourth hours after injection, and a maximum concentration was shown in the urine at the end of the third hour. In a period of 8.3 hours, 76 per cent of the oxide available for diffusion was recovered from the urine. S.T.C.

215. The Composition of the Blood and Milk of Lactating Cows during Inanition, with a Note on an Unidentified Constituent Present in Certain Samples of Abnormal Milk. J. A. B. SMITH, G. R. HOWAT AND S. C. RAY, The Hannah Dairy Research Institute, Kirkhill, Ayr. J. Dairy Res. *9*: 310–322. 1938.

The yield and composition of the milk secreted by three non-pregnant Ayrshire cows during a 12 day fast were determined. During the fast the yield fell to about one-seventh of the original figure. The protein and fat contents of the milk of all three cows rose considerably, though variably. There were marked changes in the chloride-lactose ratio in the milk from two cows with one of which inanition was complicated by milk fever and with the other by serious loss of condition. There was a marked increase in the chloride content and a decrease in the lactose content of the milk from these animals. The sum of the values obtained for protein, lactose and ash was lower than the value obtained for solids-not-fat by as much as 1.9. The authors postulate the presence of an unidentified constituent. S.T.C.